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CLINICAL PHARMACOLOGY
OF
PSYCHOACTIVE DRUGS

Edited by: E. M. SELLERS

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Edited by E. M. SELLERS

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Preface

The papers in this volume comprise the proceedings of the Symposium "Clinical Pharmacology and Toxicology of Psychoactive Drugs". This Symposium was held between October 22nd and 24th, 1973, at the Clinical Institute Addiction Research Foundation, Toronto. The symposium was one of eight International Symposia on Alcohol and Drug Research co-sponsored by the Departments of National Health and Welfare, Ottawa, Canada, and the Alcohol and Drug Addiction Research Foundation, Toronto, Canada, in association with the International Council on Alcoholism and Addictions, Lausanne, Switzerland.

The papers of this Symposium were presented during three sessions: "Etiology and Epidemiology"; "Factors Influencing Toxicity"; "Clinical and Molecular Drug Interactions". The spectrum of topics discussed was wide, ranging from the epidemiology of suicidal drug intoxication to the molecular basis of psychoactive drug action.

The purpose of the symposium was to focus on relatively unexplored areas of research, to present several important recent developments, and to discuss methodologic problems. In addition there was a deliberate attempt to intermesh basic and applied scientific approaches to related problems.

I am indebted to the people who helped in planning and conducting this symposium. I thank Drs. J.G. Rankin, E.A. Sellers, S.D. Cooper and S.M. MacLeod for their discussions about format and content and Mrs. Sylvia Lambert for her help in the editing and preparation of manuscripts for publication. I would particularly like to acknowledge the assistance of Mrs. Maureen Stewart in organizing the Symposium. It was largely because of her efforts that the Symposium was successful.

Edward M. Sellers
December 1973

Perinatal Development of Mechanisms for Drug Biotransformation

Stuart M. MacLeod, M.D., Ph.D., F.R.C.P.(C),

Kenneth W. Renton, B.Sc. and Norman R. Eade, M.D., D.Phil.

ABSTRACT A significant deficit in the concentration of cytochrome P-450 and in the activity of drug-oxidative and electron transport enzymes in the liver microsomes of newborn rats has been shown. In rats, the rate of maturational development of drug-oxidative activity appears to be limited by the development of electron transfer capacity. NADPH cytochrome P-450 reductase activity increases more gradually than microsomal cytochrome P-450 and consequently drug oxidation remains below adult levels until 5 weeks of age although cytochrome P-450 reaches adult concentration by 3 weeks of age. It has been shown that the fraction of microsomal cytochrome P-450 which is rapidly reducible *in vitro* is 50% in microsomes from 1 week old rats, less than 40% in microsomes from 2 and 3 week old rats and greater than 60% in microsomes from 4 and 5 week old rats. The deficient capacity for reduction of cytochrome P-450 may be partially overcome *in vitro* through enhancement of NADPH cytochrome P-450 reductase by a type I substrate (aminopyrine). Phenobarbital has been shown to induce all components of hepatic microsomal drug metabolism to adult levels or greater within 2 weeks of birth and this implies that the gene expression system in the newborn rat hepatocyte is complete and capable of full synthetic activity in response to appropriate exogenous or endogenous stimuli. Paradoxically, isoniazid, a hydrazine derivative which normally inhibits microsomal drug metabolism has been shown to accelerate the development of microsomal drug-metabolising enzymes in newborn rats. Measurement of the components of hepatic microsomal drug metabolism in microsomes prepared from *antemortem* human liver samples suggests that age-related deficiencies in hepatic microsomal drug oxidation and electron transport exist during infancy and during the 6th decade of life.

The study of perinatal drug metabolism remains surprisingly incomplete despite the occurrence of at least one disaster attributable to overzealous administration of drugs to premature human infants. Chloramphenicol toxicity in newborn and premature infants was first recognised in 1959[1]. The fatal syndrome included hypothermia, muscular flaccidity and an ashen-grey cyanosis which led to the designation "grey syndrome". Mortality correlated directly with immaturity and chloramphenicol administration proved fatal to at least 50% of premature infants receiving the drug.[2, 3] At least in part, this mortality is explained by underdevelopment of biotransformation mechanisms in inmature infants.

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It is important to determine the *functional* status of hepatic microsomal drug-metabolising enzymes during the newborn period in order to identify rate-limiting factors in the development of full activity and to avert further therapeutic misadventures. At present, there are few facts available about the development of hepatic drug-oxidizing enzymes in humans because of practical difficulties in obtaining large quantities of normal human liver and as a result it has been necessary to approach this problem by studying maturational development of drug-biotransformation in laboratory animals.

DEVELOPMENT OF HEPATIC MICROSOMAL DRUG METABOLISM IN NEWBORN LABORATORY ANIMALS

Fouts and Adamson, in 1959, found that newborn rabbits were essentially unable to metabolise a variety of drugs including hexobarbital, aminopyrine, amphetamine, and chlorpromazine.[4] Drug-oxidative activity in most pathways rose to near adult levels at 4 weeks of age. Subsequently, numerous authors including ourselves, have documented the underdevelopment of drug metabolising enzymes in newborn animals.[5-15]

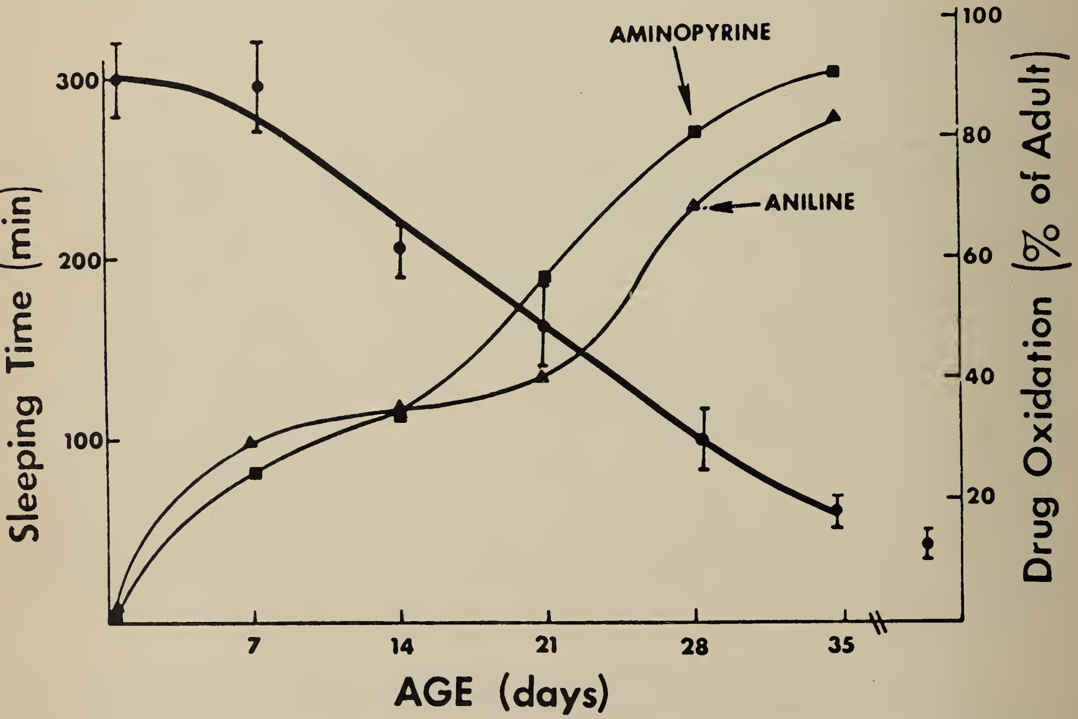

Figure 1. *Normal maturational development of hepatic microsomal drug-oxidation measured* in vitro *and* in vivo *in male rats of varied age. The heavy line represents the decreasing duration of pentobarbital-induced sleep in response to a pentobarbital dose of 30 mg/kg in contrast to the age-related increase in hepatic microsomal drug-oxidative activity. Each sleeping time point is the mean ± S.E. of 10 determinations. Mean specific activities of aminopyrine N-demethylase and aniline p-hydroxylase are presented as a percentage of mean specific activities observed in preparations of liver microsomes from adult male rats.*

Figure 1 shows the normal maturational development of hepatic microsomal drug oxidation measured *in vitro* and *in vivo* in male rats of varied age. It may be seen that the duration of pentobarbital-induced sleep in response to a pentobarbital dose of 30 mg/kg decreased progressively as age increased. This decreasing duration of pentobarbital-induced sleep is in direct contrast with the age-related inversely proportional increase in hepatic microsomal drug-oxidative activity as measured in the specific activity of aminopyrine N-demethylase, and aniline p-hydroxylase activity shown in Figure 1 as a percentage of their adult activities.

Figure 2 shows the progressive maturational increase in aminopyrine N-demethylase, cytochrome P-450 and NADPH cytochrome P-450 reductase. When all of these values are graphed as a percent of normal adult levels, it is seen that the progressive increase in aminopyrine N-demethylase activity closely parallels the increasing activity of NADPH cytochrome P-450 reductase but lags behind the more rapid increase in microsomal cytochrome P-450 content. This suggests that the development of electron transport mechanisms is rate-limiting to the development of full drug-oxidative activity during maturation. These results, which have been reported by us elsewhere,[15] confirm the earlier report by Gram, *et al.*[16]

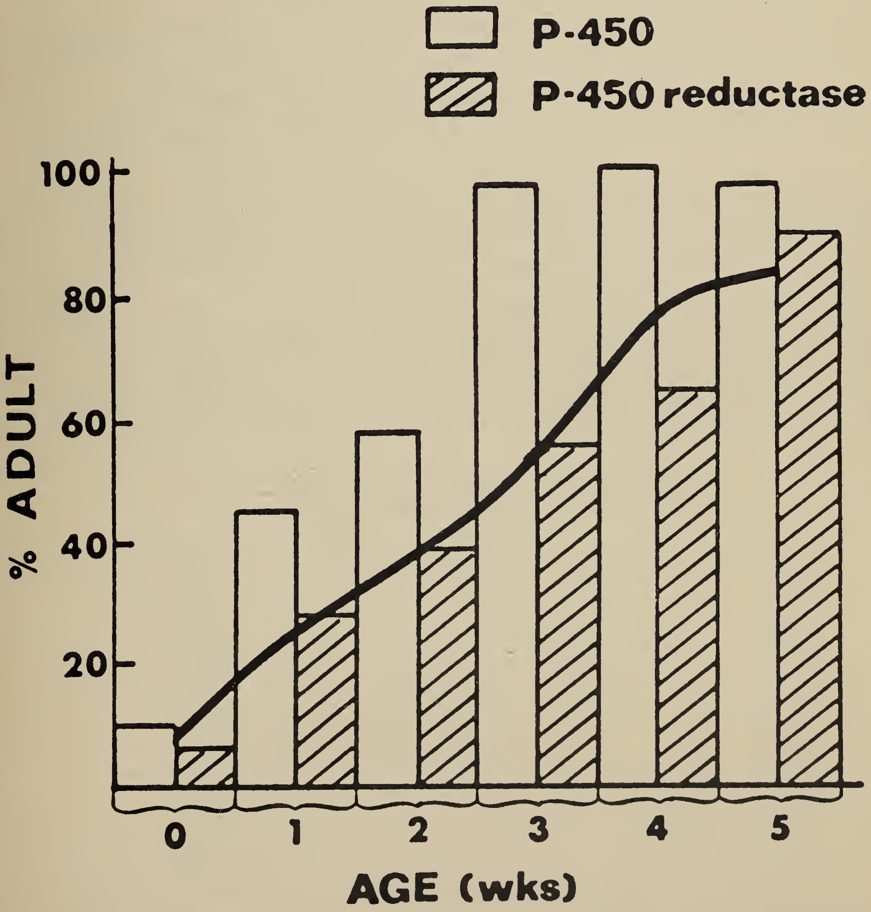

Figure 2. *Normal maturational increase in cytochrome P-450 content (open bars), NADPH cytochrome P-450 reductase activity (hatched bars) and aminopyrine N-demethylase activity (solid line) in liver microsomes from male rat pups. Values at each age are expressed as a percentage of normal mean adult values.*

DEVELOPMENT OF DRUG BIOTRANSFORMATION MECHANISMS IN NEWBORN HUMANS

Despite the problem in obtaining human liver samples, it has in the last 4 years been shown that cytochrome P-450, NADPH cytochrome c reductase, NADPH cytochrome P-450 reductase and cytochrome b5 are all present in normal human hepatic microsomes.[17, 18, 19, 20] There remains, however, an almost complete lack of information about the normal development of drug biotransformation mechanisms *in utero* or in early life. In 1970, Yaffe and his co-workers obtained fetal livers at elective abortion and studied the drug-metabolising capacity of 13 human fetuses ranging in gestational age from 14 to 25 weeks,[21] Surprisingly, despite a time lapse of at least 5 hours between abortion and preparation of liver homogenates, these authors found measurable levels of all components of the drug-metabolising enzyme system. It was also found that exogenous substrates were not consistently or actively oxidized while endogenous substrates such as testosterone were oxidized at rates comparable to those found in preparations of adult rat hepatic microsomes. In our own laboratory, we have studied the biochemistry of human drug-metabolising enzymes *in vitro* using the most normal biopsy specimens so far obtainable from patients of varied age ranging from 4 days to 60 years. The specimens came from the surgery department of the Montreal Children's Hospital and from the renal transplantation service of the Royal Victoria Hospital in Montreal. Because of the limited amounts of human tissue available in some cases, it was not possible to perform the complete spectrum of biochemical assays on all samples.

Figure 3 shows the specific activities of aminopyrine N-demethylase measured in 9 human liver samples. Activity was very low in samples from 4 day and 8 day old infants and was considerably higher than average in samples from 20 and 21 year old subjects. The activity in only 1 human sample, that of a 20 year old male renal transplant donor, approached the activities normally found in adult male rats. There appears to be approximately a threefold difference in activity between activities measured in 20 and 21 year old subjects and those found in 59 and 60 year old subjects.

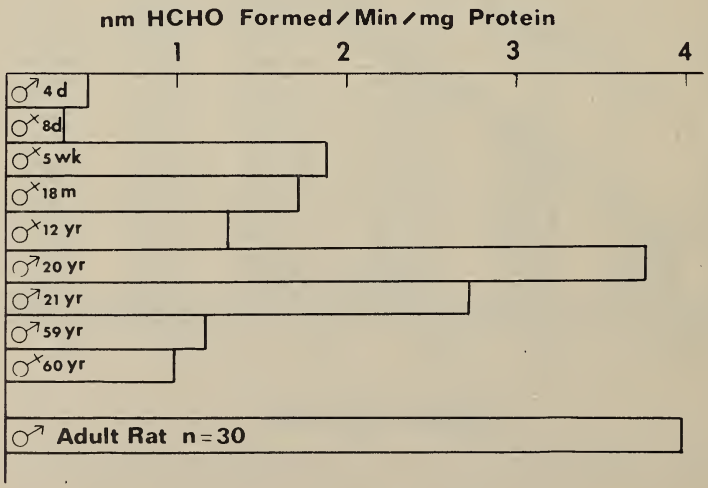

Figure 3. *Specific activity of aminopyrine N-demethylase in liver microsomes from 9 humans of varied age.*

Figure 4 shows the specific activities of aniline p-hydroxylase measured in the liver samples from the same 9 subjects. There was relatively little variation among these samples except for the markedly higher activity in liver microsomes from 20 and 21 year old subjects. The liver microsomes from 4 and 8 day old infants were not markedly deficient in aniline-hydroxylating activity relative to most of the remaining age range.

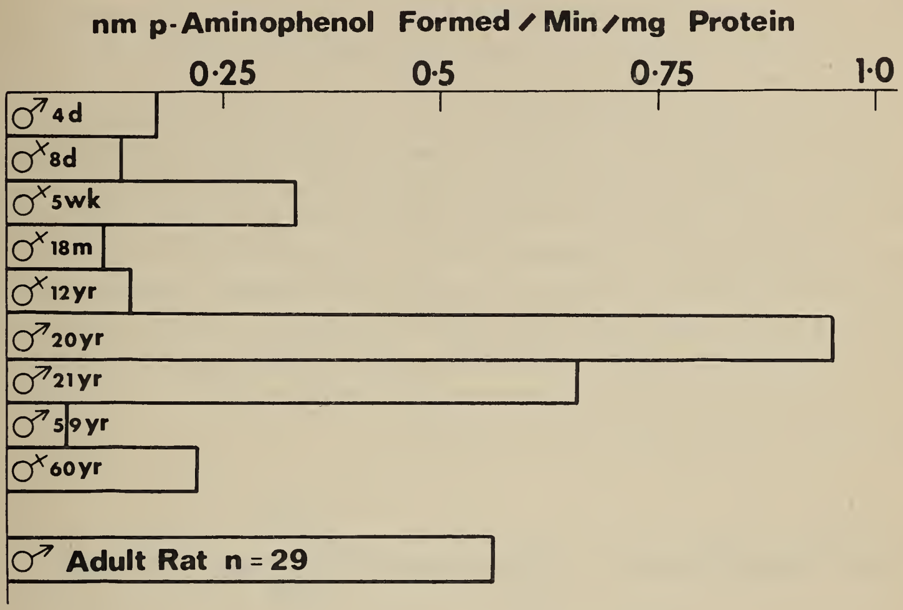

Figure 4. *Specific activity of aniline p-hydroxylase in liver microsomes from 9 humans of varied age.*

Figure 5 shows the microsomal concentration of cytochrome P-450 in preparations from 10 human liver samples. All samples showed concentrations of the hemoprotein considerably lower than those found in the adult rat. The cytochrome P-450 concentration was somewhat lower than average in samples from patients aged up to 4 months; however only at 4 months was the content strikingly deficient.

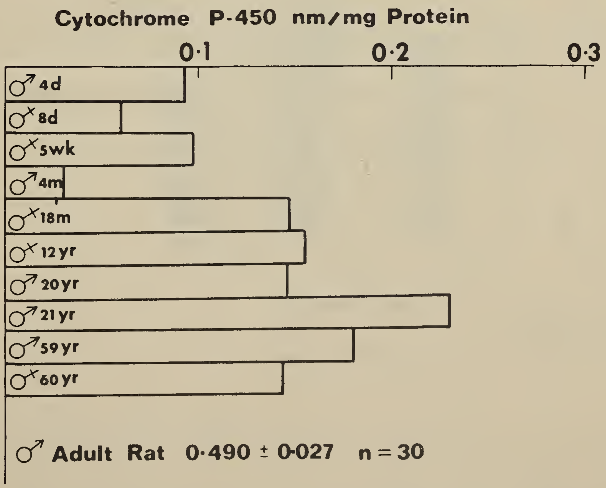

Figure 5. *Concentration of cytochrome P-450 measured in liver microsomes from 10 humans of varied age.*

Figure 6 shows the specific activity on NADPH cytochrome c reductase measured in liver samples from 9 humans of varied age. The electron transfer flavoprotein was quite active in samples from 4 day old, 8 day old and 5 week old infants and only the 4 month old infant was strikingly deficient in this regard. It is noteworthy that the electron flux as indicated by the enzyme's activity was quite low in microsomes prepared from biopsy material of 59 and 60 year old patients.

These data are preliminary and do not permit detailed interpretation; however, it has been shown that there is some degree of drug-oxidative activity in microsomes prepared from human infant liver samples and this finding is in accord with Yaffe's work.[21] There were substantially higher levels of activity in microsomes from 20 and 21 year old patients and this suggests that the drug-metabolising system may gradually increase in functional capacity over a prolonged period, perhaps the first half of a normal life span. Substrate oxidation had declined in the 6th decade suggesting that there are age-dependent variations in drug metabolism at both extremes of life.

The electron transport pathway associated with human drug metabolism appears relatively well developed in infants but lower activity of NADPH cytochrome c reductase in hepatic microsomes from 59 and 60 year old patients suggests that this aspect of drug-metabolic function may decline significantly with age and lead to a diminished capacity for drug biotransformation.

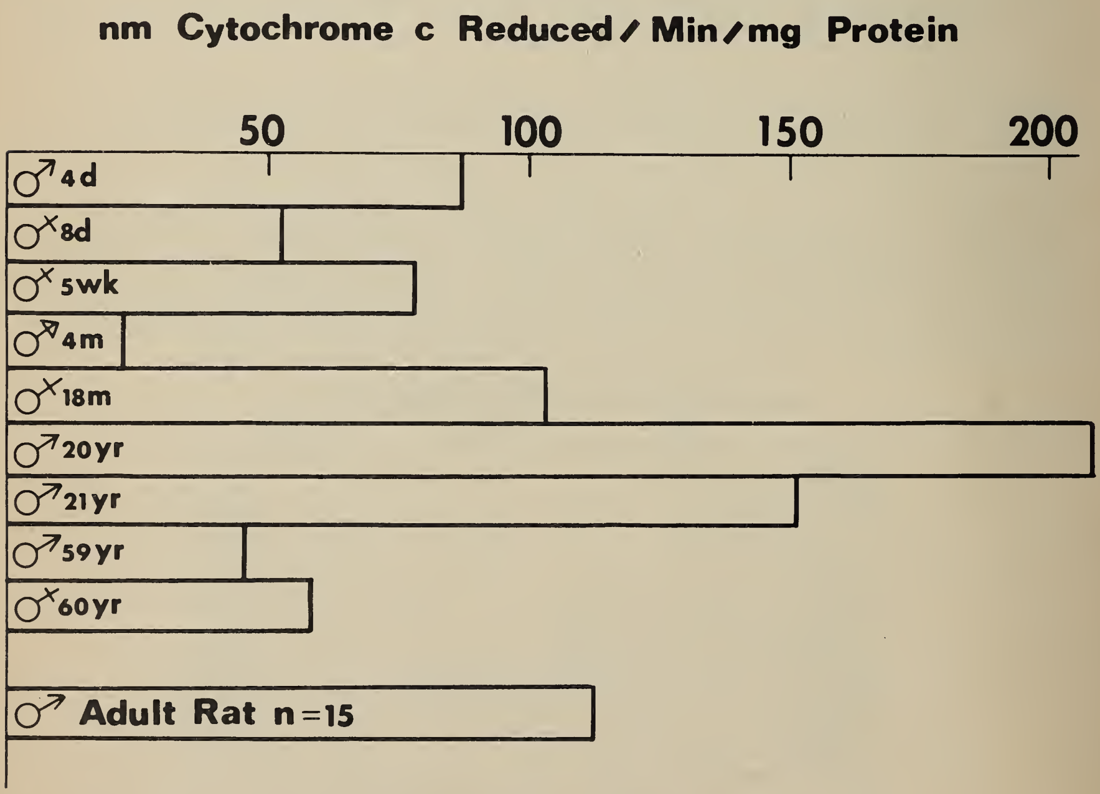

Figure 6. *Specific activity of NADPH cytochrome c reductase measured in liver microsomes from 9 humans of varied age.*

ANTE AND POST NATAL DRUG EFFECTS ON THE DEVELOPMENT OF HEPATIC MICROSOMAL DRUG OXIDATION

Pediatricians have long been interested in the possibility of augmenting newborn biotransformation of bilirubin in order to prevent kernicterus. Maurer and his co-workers[22] showed in 1968 that phenobarbital given to pregnant women in a dose of 60 mg. daily for 2 weeks prior to delivery resulted in 50% reduction in mean serum bilirubin during the first four neonatal days. This reduction in expected hyperbilirubinemia was attributed to induction of bilirubin-conjugation mechanisms in liver microsomes. Hart and his co-workers showed in 1962 that gestational treatment of female rabbits prior to the last seven days caused no change in the functional status of mixed function oxidase in their offspring.[23] Similarly, Fouts in 1968 reported that phenobarbital was an effective inducing agent in rabbits only when given during the last four days of gestation.[24] It appears that once the hepatocellular gene action system is complete, all that is required for full expression of microsomal drug oxidative activity is an endogenous trigger which normally acts after birth or an exogenous trigger such as phenobarbital which is able to work in the late fetal period.

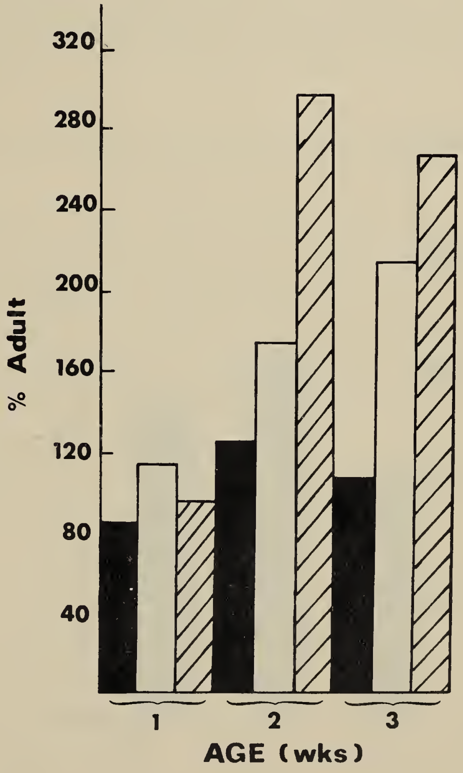

Figure 7. *Inducibility by phenobarbital of hepatic microsomal aminopyrine N-demethylase (black bars), cytochrome P-450 (open bars) and NADPH cytochrome P-450 reductase (hatched bars) in immature male rats. Mean specific activities of enzymes and concentrations of cytochrome P-450 are expressed as a percentage of normal adult male values.*

We have examined the effects of phenobarbital given to rats during the newborn period on the maturational development of all components of the hepatic microsomal mixed function oxidase system. Figure 7 shows the inducibility by phenobarbital of hepatic microsomal aminopyrine N-demethylase, cytochrome P-450, and NADPH cytochrome P-450 reductase in newborn rats. When values of all these parameters are expressed as a percentage of normal adult male values, it is apparent that there is greater augmentation by phenobarbital of NADPH cytochrome P-450 reductase and of cytochrome P-450 content than there is of drug oxidation.

Figure 8 demonstrates the perinatal inducibility by phenobarbital of hepatic microsomal aminopyrine N-demethylase and of NADPH cytochrome c reductase. It is clear that there is a close parallel in the degree to which these enzymes are induced by phenobarbital at all ages studied, suggesting that the inducibility of NADPH cytochrome c reductase might determine the degree to which drug-oxidative activity may be induced. The induced values for drug oxidation are more meaningfully compared with normal values in animals of corresponding age and this comparison is made in Figure 9 which shows the pattern of development of specific activity of aminopyrine N-demethylase in untreated and phenobarbital treated male rats from birth to 3 weeks of age. After male rat pups had received 1 week of treatment with phenobarbital, the specific activity of aminopyrine N-demethylase was increased threefold over normal. Maximal induction of aminopyrine N-demethylase was observed at 2 weeks of age.

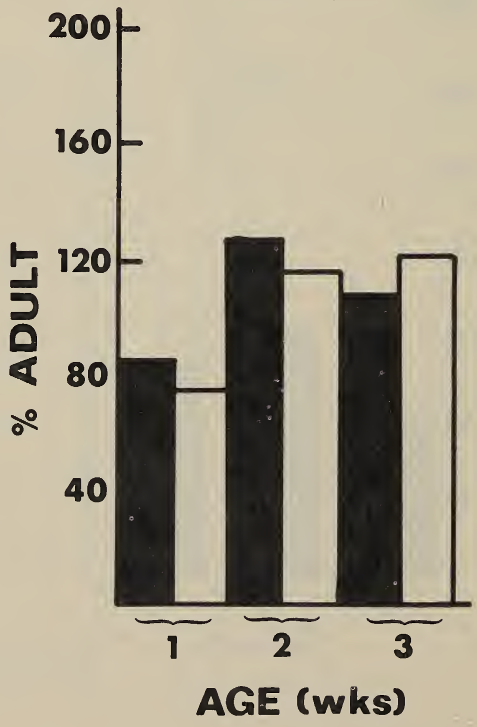

Figure 8. *Inducibility by phenobarbital of hepatic microsomal aminopyrine N-demethylase (black bars) and NADPH cytochrome c reductase (open bars) in immature male rats. Mean specific activities are expressed as a percentage of normal adult male values.*

These results with phenobarbital treatment of immature rats indicate that the gene-action system of the newborn rat hepatocyte is complete by one week of age and lacks only a physiological trigger to stimulate enzymatic differentiation and the rapid attainment of full expression of drug-metabolising enzyme activity. Increases in drug metabolism beyond adult levels were not observed in these experiments with the dose of phenobarbital used, suggesting that such excess stimulation required either hepatocellular or extrahepatic factors which were not available during the newborn period.

After demonstrating these effects of phenobarbital, a known inducer of microsomal drug metabolism, we examined the effects of isoniazid, a hydrazine derivative known to inhibit hepatic microsomal drug metabolism both *in vivo* and *in vitro*.[25, 26, 27, 28] Isoniazid was given in a dose of 10 mg/kg to pregnant females and to newborn male rats. Paradoxically, this normally inhibitory drug was found to accelerate the development of microsomal drug metabolising enzymes. Figure 10 compares the normal and isoniazid-stimulated maturational increase in mean specific activity of hepatic microsomal aniline p-hydroxylase in male rats. When isoniazid was given postnatally, the mean specific activity of aniline p-hydroxylase exceeded control values at all ages from 1 to 4 weeks. When isoniazid was given antenatally, the enzyme activity was significantly augmented at 1 and 3 weeks of age.

It is difficult to interpret the stimulatory action of isoniazid on drug-metabolising enzymes of immature male rats but further investigation of the phenomenon might clarify mechanisms which normally trigger the onset of enzymic differentiation.

ELECTRON TRANSPORT IN DEVELOPING HEPATIC MICROSOMAL MIXED FUNCTION OXIDASES

It has been demonstrated (*vide supra*) that the progressive maturational increase in aminopyrine N-demethylase activity in rats closely parallels the increasing electron transport activity of NADPH cytochrome P-450 reductase and does not correlate with increasing microsomal content of cytochrome P-450. More detailed studies of the development of microsomal electron transport activity and specifically of NADPH cytochrome P-450 reductase are presented in Figure 11. NADPH cytochrome P-450 reductase activity was determined by following the absorbance change at 450 nm when carbon monoxide-saturated microsomes were reduced by NADPH in a modification of the method described by Gigon *et al.*[29] Double exponential plots of the activity of NADPH cytochrome P-450 reductase were prepared in the manner described by Gigon and his co-workers.[30] The percentage of total microsomal cytochrome P-450 content which remained unreduced *in vitro* ten seconds after the addition of NADPH is plotted against age. The increase in this percentage at 2 and 3 weeks of age to 72 and 68% respectively is indicative of the relative inefficiency of microsomal electron transport at those ages. Although 1 week old animals had a significantly lower microsomal content of cytochrome P-450 (see Figure 2), they were relatively more able to reduce the cytochrome so that less than 60% remained unreduced 10 seconds after the addition of NADPH. The lower curve in Figure 11 demonstrates aminopyrine enhancement of NADPH cytochrome P-450 reductase activity. This is a well known effect of type I substrates *in vitro*.[29] Although a greater percentage of total cytochrome P-450 was reduced at all ages, the pattern of development of the reductase was unchanged and the relative deficiency of electron transport in animals 2 and 3 weeks of age was again observed.

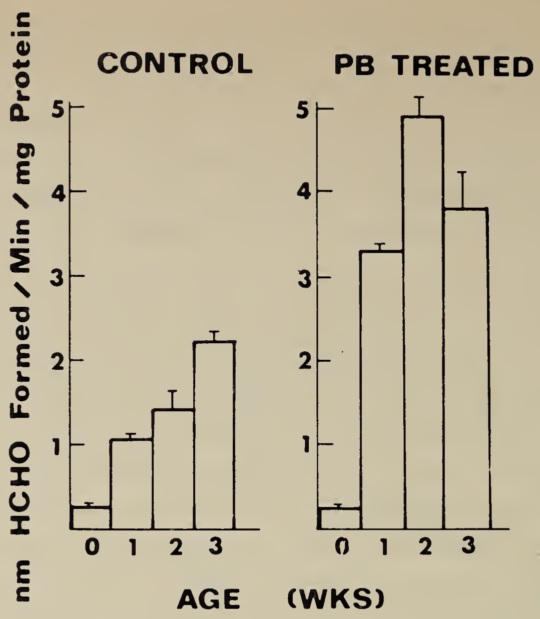

Figure 9. *Comparison between the normal and phenobarbital-stimulated maturational increase in the mean specific activity of hepatic microsomal aminopyrine N-demethylase in male rats. Each bar represents the mean activity ± S.E. at a given age. Normal adult specific activity for demethylation of aminopyrine (3.99 ± 0.25 nanomoles of formaldehyde formed per minute per mg protein) is exceeded by 2 weeks of age in microsomes from phenobarbital-treated rats.*

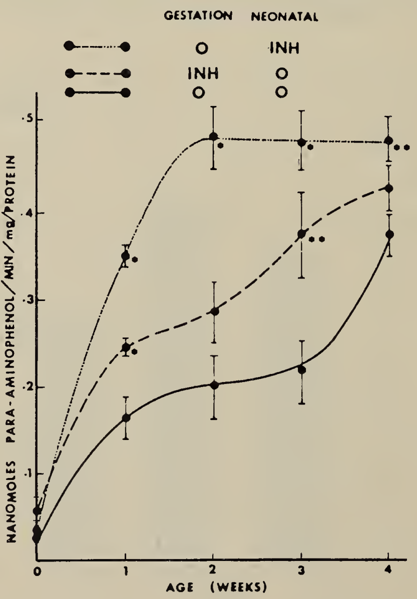

Figure 10. *Comparison between the normal (solid line) and isoniazid-stimulated maturational increase in mean specific activity of hepatic microsomal aniline p-hydroxylase in male rats. Points are represented ± their S.E. When isoniazid was given postnatally (dotted line) the mean specific activity of aniline p-hydroxylase exceeded control values at all ages from 1 to 4 weeks (*p<.01;**p<.05). When isoniazid was given antenatally (dashed line) the enzyme activity was significantly augmented at 1 and 3 weeks of age (*p <.01;**p <.05).*

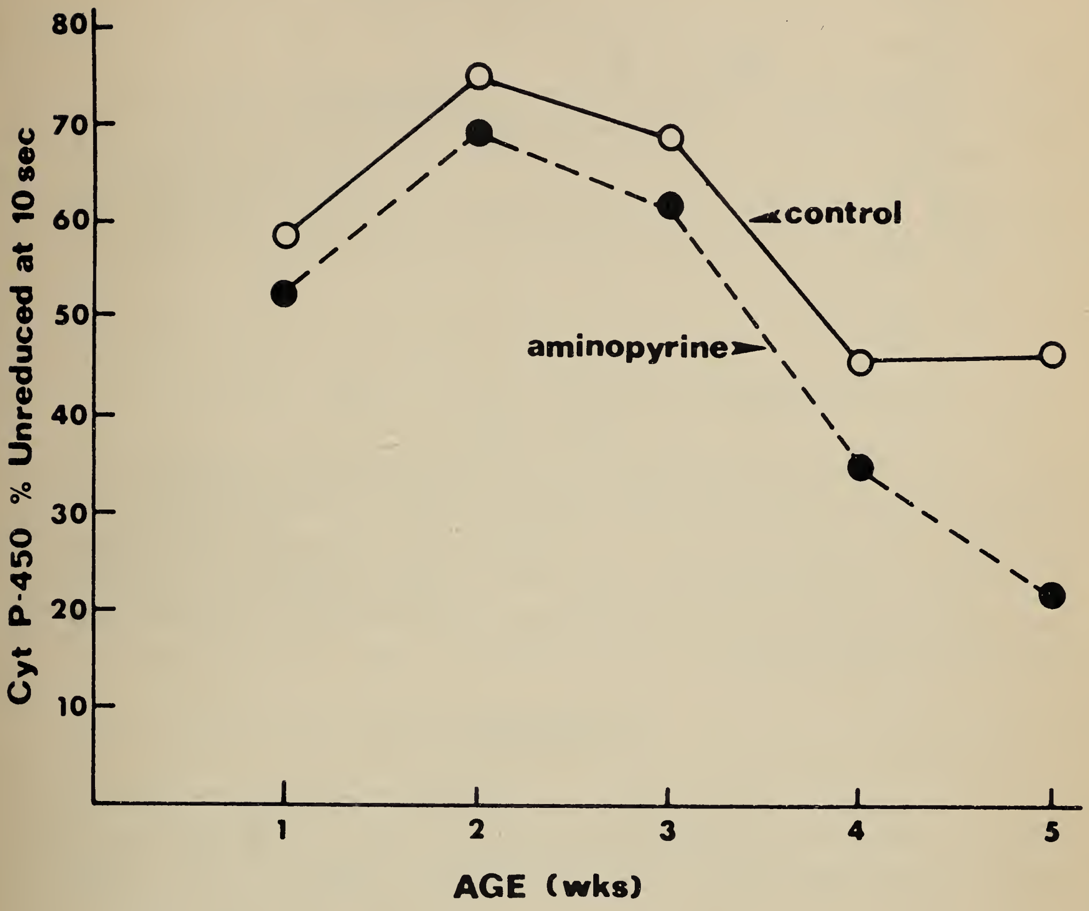

Figure 11. *Percentage of total cytochrome P-450 remaining unreduced 10 seconds after the addition of NADPH* in vitro *to liver microsomes from male rat pups of varied age in the presence and absence of aminopyrine (concentration of 1 mM).*

This deficiency in electron transport activity is clarified in Figure 12 which shows the maturational change in the concentration of rapidly reducible cytochrome P-450 in liver microsomes from male rat pups. The percentage of total microsomal cytochrome P-450 which is rapidly-reducible is an indicator of the facility of electron flux within the microsomal system. In Figure 12, all values for rapidly-reducible cytochrome P-450 are expressed as a percentage of total cytochrome P-450 content at a given age. Until 4 weeks of age under control conditions, there is, relative to adult, a lower proportion of rapidly-reducible cytochrome P-450 present in liver microsomes and the deficiency is most pronounced at 2 and 3 weeks of age. When aminopyrine is added to a concentration of 1 millimolar there is an increase in the proportion of rapidly reducible cytochrome P-450 at 2 and 5 weeks of age.

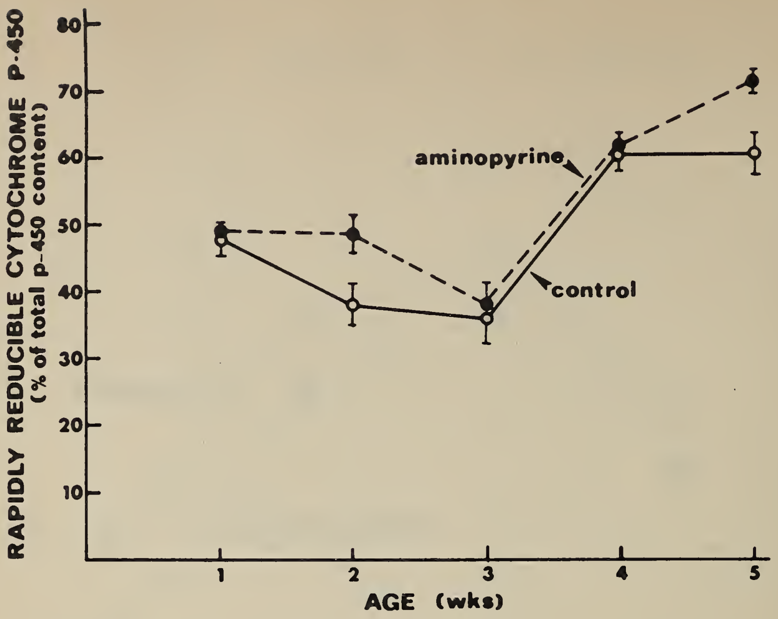

Figure 12. *Pattern of maturational change in the concentration of rapidly-reducible cytochrome P-450 in liver microsomes in male rat pups. All values are expressed as a percentage ± the standard error of total microsomal cytochrome P-450 content at the given age. When aminopyrine was added to a concentration of 1 mM (dashed line) there was an increase in the proportion of rapidly-reducible cytochrome P-450 at 2 and 5 weeks of age.*

CONCLUSIONS

The results presented indicate several problems and show essential directions for research in this area. It is quite apparent that we need to determine the normal activity of all components of drug metabolism in humans of varied age and to ascertain whether the functional status of the biotransformation system is seriously compromised in the newborn period or with advancing age. The need for such information is pressing because of the current use in neonatology of drugs such as diphenylhydantoin, phenobarbital, chloramphenicol and diazepam, all of which undergo hepatic biotransformation. Changing biotransformation characteristics with increasing age are of interest because of greater requirements for drug therapy in the geriatric population. Furthermore, it should be remembered that these same microsomal enzymes are involved in the metabolism of environmental hydrocarbons and of endogenous steroid hormones so that the implications of age-related variation in enzyme activity go far beyond the area of drug metabolism.

The data presented demonstrate that the maturational development of drug oxidative activity is most closely linked in rats to the full maturational development of mechanisms for microsomal electron transport and is not directly related to the microsomal content of cytochrome P-450. It is therefore interesting to speculate that the safety of drug therapy in newborns might be enhanced by the augmentation of electron transfer activity. Such augmentation might be achieved by the selective use of drugs which are Type 1 substrates and thus are able to facilitate electron transfer and drive their own biotransformation, or by the discovery of other substances which are not necessarily substrates of the mixed function oxidase system but which have the capacity to increase electron transfer and drive the metabolism of simultaneously administered therapeutic agents.

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Perinatal Toxicology: The Question of Delayed and Residual Effects

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ABSTRACT The potential for delayed and (or) residual effects, including toxicity, with use of pharmacological agents in the perinatal period is emphasized. With use of data from the literature and from our laboratory, the existence, subtlety and complexity of such events is first discussed in terms of perinatal "imprinting" by androgens. The importance of brief, neonatal exposure to testosterone for subsequent adult responsiveness of rat seminal vesicle and coagulating gland to androgen is described. Ventral prostate responds differently, without "imprinting" by testosterone, but with response to organic extracts of neonatal testes. Similar experiments encompassing neonatal castration and androgen replacement reveal distinct influence on the ontogenetic pattern of rat hepatic aniline hydroxylase activity. Neonatal, as against later, castration produced no unique effects with respect to aminopyrine demethylase. Surprisingly, the aminopyrine demethylase activities of early, but not late, castrates lose responsiveness to androgen at established maturity.

Certain aspects of perinatal brain development are reviewed in the context of susceptibility to toxic modification. The effects on brain development of perinatal irradiation, cyclophosphamide, methylazoxymethanol, cycasin, 6-hydroxydopamine and its analogs, methamphetamine, chlorpromazine, and thyroid hormone are reviewed.

The purpose of this report is to stress the potential, even probable, significance of the permanent, subtle effects resulting from interaction between pharmacologic or toxic agents and the perinatal organism. With respect to acute toxicity, there is ample evidence[1] to conclude that the perinate, as against the adult, exhibits qualitatively and quantitatively distinct responses to drugs, probably as the result of one or a combination of differences in absorption, distribution, metabolism, excretion and target organ sensitivity. The "gray-baby" syndrome associated with the perinatal administration of chloramphenicol can be cited as an example.[2] Our concern herein is not to expound upon such distinctive aspects of acute toxicity, but rather, to emphasize the potential for residual and/or delayed effects, toxic or otherwise, due to insult in the perinatal period.

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Residual and delayed toxicity in the earlier stages of development, before the perinatal period, is often gross. One can cite the well known teratologic effects of thalidomide[3] and the vaginal adenocarcinoma associated with diethylstilbesterol.[4] Residual or delayed toxicity due to insult in the perinatal period is likely to be more subtle although the staining of teeth by tetracyclines is not.[5]

Significantly, the perinatal period is not fixed in time. Since the events of labour, delivery and neonatal illnesses result in exposure of the infant to a large group of pharmacological agents, intentionally or accidentally (e.g., transplacental or with lactation), it seems appropriate here to define "perinatal" not as the interval surrounding delivery after normal gestation, but as the period surrounding actual delivery. In view of the fact that many more very low-birth-weight infants are now surviving, it seems reasonable to maintain flexibility in definition. Indeed, in the context of gestational time, the earlier the perinatal period, the more blatant are the effects we are likely to encounter.

We discuss firstly the "imprinting" influence of sex hormones in the perinatal period to establish both the validity and the complexity of this subject matter. In the context of this Symposium, the influence of neuropharmacologic agents on the perinatal brain must be evaluated most carefully, on the one hand, because of the important ontogenetic events occurring late in gestation and postnatally (e.g., synaptogenesis and myelinogenesis) and, on the other hand, because of the clinical difficulties encountered in the assessment of even gross insult to brain at this time. Therefore, we include a brief description of certain aspects of the perinatal ontogenesis of the brain and conclude with an enumeration of certain agents which, when administered in the perinatal period, provoke permanent, deleterious effects upon brain.

IMPRINTING BY SEX HORMONES

Conceptualization of the perinatal period as "critical", in the developmental and teratologic sense, rests in significant measure on studies involving the action of sex hormones,[6,7] as well as corticosteroids[8] and thyroxine.[9] These compounds can act to alter permanently the course of differentiation of the brain and other hormone-sensitive target tissues. Actions of these chemical inducers are characterized by their irreversibility. Their effects tend to persist throughout life despite the inability to detect the original inducer(s).

The timing and duration of the "imprinting" period depends probably on the species, the inducer, the target tissue, and the assayed action. Although many of the effects described below are observed postnatally in rodents, most are likely to occur prenatally in man. Nonetheless, the escalating survival of very low-birth-weight infants makes it increasingly likely that certain "imprint" situations do or will fall within the perinatal period. Indeed, certain current practices with low-birth-weight infants make these considerations of more than experimental interest: as examples, the perinatal administration of corticosteroid to prevent respiratory distress syndrome,[10] and the use of compounds such as phenobarbital with inducing capacity for the purpose of accelerating the elimination of bilirubin.[11]

Discussion of the action of sex hormones is divided according to target: sex accessory organs and liver. No attempt has been made to be encyclopedic; rather, exemplary experiments are discussed. Neonatal imprinting of the brain, particularly of the hypothalamus, for behaviour and positive feedback is not discussed herein, but constitutes a related area of significant interest and investigation.

Sex Accessory Organs

The seminal vesicles of adult rodents that had been castrated on the first day of life do not respond to the administration of testosterone nearly as well as seminal vesicles of animals that had been similarly castrated but given testosterone on days 2 and 4 after birth. Effective "imprinting" for subsequent response to testosterone is accomplished only when androgen is administered in the first few days of post-natal life.[12, 13] Notably, the basal weight and DNA content of adult seminal vesicles after neonatal castration with and without neonatal androgen replacement are identical; only the response to testosterone differs. It is not clear whether a variety of sex accessory organs (seminal vesicles, ventral prostate and coagulating gland) will respond to the same degree to the neonatal "imprinting" effects of androgens.

Male rats were castrated within 24 hours of birth. Some received testosterone propionate (500 μg/rat) in corn oil at age 2 and 4 days; controls were treated with corn oil. At puberty, the degree of androgen responsiveness re the growth of the sex accessory tissues was measured. Parameters of response included the determination of net wet weight, DNA, RNA and protein content. The responsiveness of adult rats that had been castrated 10 days earlier was used for comparison (Tables I and II). The initial DNA content of each sex accessory organ was highest in the 10-day adult castrates. At the end of testosterone stimulation, the net accumulation of wet weight, DNA, RNA and protein content is also the highest in 10-day adult castrates as against those animals castrated earlier in life. Similar to the findings in mice,[12, 14] neonatal administration of androgen enhanced the response of seminal vesicles to adult androgen stimulation 60-70% beyond that of the non-androgenized group. Neonatal androgenization appears to exert the same effects on the coagulating glands but only stimulates marginally (20%) the response of ventral prostate (Tables I and II). The "permanent" change in both seminal vesicle and coagulating gland resulting from neonatal castration is reflected also by a significantly lower protein/DNA ratio in these glands after testosterone stimulation (Table I). Under the same conditions, the protein/DNA ratios of the ventral prostate are the same as those exhibited by the 10-day adult castrates (Table II).

Differences in the developmental aspects of ventral prostate as compared to seminal vesicle and coagulating gland were further evaluated by two additional types of experiments. Firstly, the "imprinting" effects of neonatal androgens have been visualized as the "unloading" of testosterone from testes during neonatal period. If this was the case, then the organic extract from these pooled newborn testes could mimic the action of testosterone as described above. Experimental results of these studies indicate that the crude organic extract from newborn testes only increases the response of ventral prostate to adult androgen stimulation. Minimal or no effects on the responses of seminal vesicle and coagulating gland were observed (Figure 1). It is difficult to explain why the crude testicular extract only "imprints" for the response of ventral prostate but not seminal vesicle and coagulating gland, the inverse of neonatally administered testosterone. Nevertheless, these experiments reveal that ventral prostate differs from seminal vesicle and coagulating gland in imprinting characteristics and serves to further emphasize ignorance of such events. Interestingly, in 1936, Price[15] showed that the microscopic anatomy of seminal vesicles from adult rats was arrested in its immature stage when castration was performed in the neonatal period. Under identical conditions, only minimal changes were seen in the prostate gland.

TABLE 1.

EFFECTS OF NEONATAL ANDROGEN ON THE RESPONSIVENESS OF SEMINAL VESICLE (SV) AND COAGULATING GLAND (CG) TO ADULT TESTOSTERONE TREATMENT

Animal* (body weight)	Gland	Wet weight of gland (mg/100 g b.w.)	DNA content (µg/100 g b.w.)	Ratio Protein DNA mg/mg
		Oil/TP†	Oil/TP†	Oil/TP†
Adult Castrate (349 ± 20)	SV	40/135±12	120/371±84	–/38±4
	CG	14/50±2	18/65±8	–/74±9
Newborn Castrate + Oil (346 ± 18)	SV	2.5/31±2	25/91±14	15/27±1
	CG	–/12±1	–/27±4	–/32±3
Newborn Castrate + TP (365 ± 13)	SV	2.9/60±7	24/152±14	15/30±4
	CG	–/21±3	–/42±3	–/39±2
Newborn Castrate + Neonatal Testicular Extract (346 ± 6)	SV	1.7/37±3	13/110±15	–/26±3
	CG	–/14±1	–/26±4	–/27±6

*Rats were either castrated on the day of birth (newborn) or at age 120 days (adult). The newborn castrates were treated with corn oil, testosterone proprionate (500 µg/rat/day) in corn oil, or ethyl acetate extract of neonatal testis in corn oil, on days 2 and 4 of life.

†All animals received a 10-day-course of testosterone proprionate (TP), 2 mg/rat/day, s.c., in corn oil or corn oil alone beginning at age 130 days. Data represent the average for 3 rats, or average from 4 to 8 rats ± S.E.M.

TABLE II

EFFECTS OF NEONATAL ANDROGEN ON THE RESPONSIVENESS OF VENTRAL PROSTATE TO ADULT TESTOSTERONE TREATMENT

	Wet weight of gland (mg/100 g b.w.)	DNA content (μg/100 g b.w.)	Ratio Protein DNA mg/mg
Animal* (body weight)	Oil/TP†	Oil/TP†	Oil/TP†
Adult Castrate (349 ± 20)	18/87±6	55/138±15	–/40±2
Newborn Castrate + Oil (346 ± 18)	2.9/53±5	17/92±4	20/41±3
Newborn Castrate + TP (365 ± 13)	3.9/65±9	24/110±13	17/37±2
Newborn Castrate + Neonatal Testicular Extract (346 ± 6)	2.1/80±5	14/155±13	21/28±3

Secondly, since neonatal testicular androgens act only at a certain discrete period of time, the possible effects of delayed testectomy on the response of sex accessory tissues to adult androgen stimulation were evaluated. Figure 2 shows that delaying castration minimizes in a graded fashion the decrease in responsivity of seminal vesicle and coagulating gland to the adult androgen; there is no effect on the responsiveness of ventral prostate.

Since androgens are known to stimulate the growth of sex accessory tissues in both immature and mature castrated rats, it is possible that neonatal androgen either enhances cell division or prevents the regression of cells in neonatal sex accessory glands. These cells might account for the increased responsiveness of seminal vesicles to later adult injections of testosterone. This possibility was studied by *in vivo* pulse

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*Rats were either castrated on the day of birth (newborn) or at age 120 days (adult). The newborn castrates were treated with corn oil, testosterone proprionate (500 μg/rat/day) in corn oil, or ethyl acetate extract of neonatal testis in corn oil, on days 2 and 4 of life.

†All animals received a 10-day-course of testosterone proprionate (TP), 2 mg/rat/day, s.c., in corn oil or corn oil alone beginning at age 130 days. Data represent the average for 3 rats, or average from 4 to 8 rats ± S.E.M.

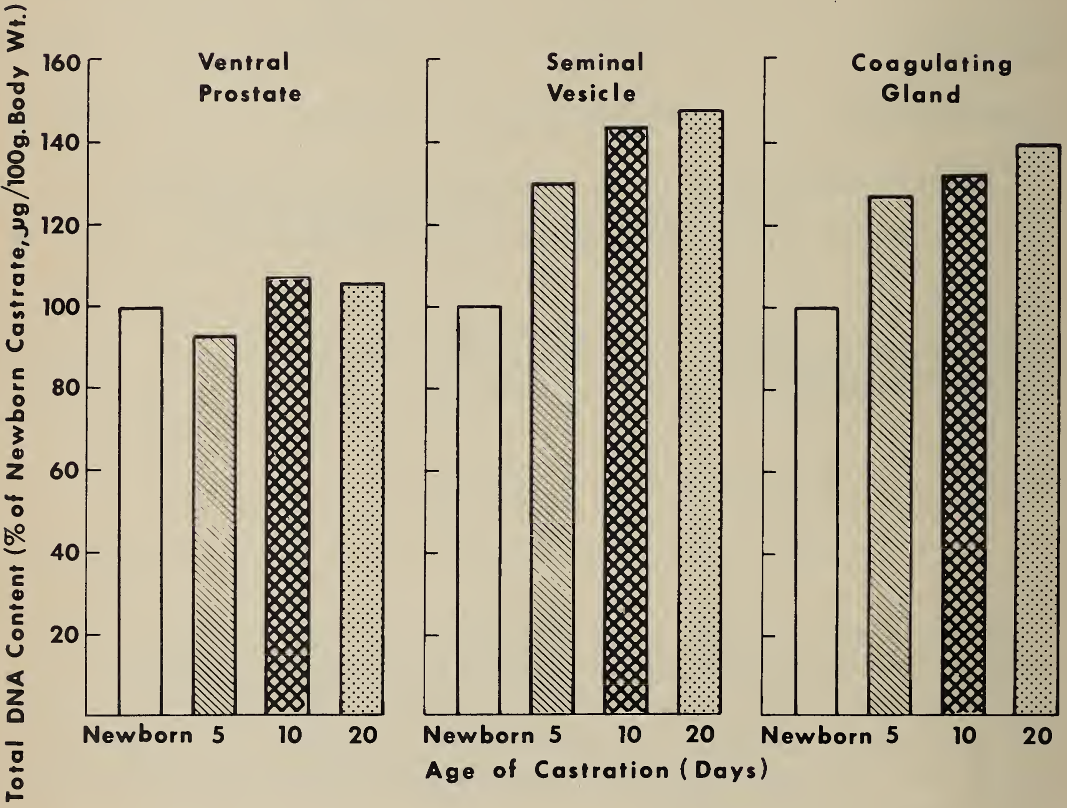

Figure 1. *The influence of neonatally-administered testosterone propionate (TP) and organic testicular extract on modifying the adult responsiveness of the sex accessory organs to TP. All rats received TP, 2 mg/rat/day, for 10 days before sacrifice at age 130 days. Results are expressed in comparison with neonatal castrates which had received corn oil (100%).*

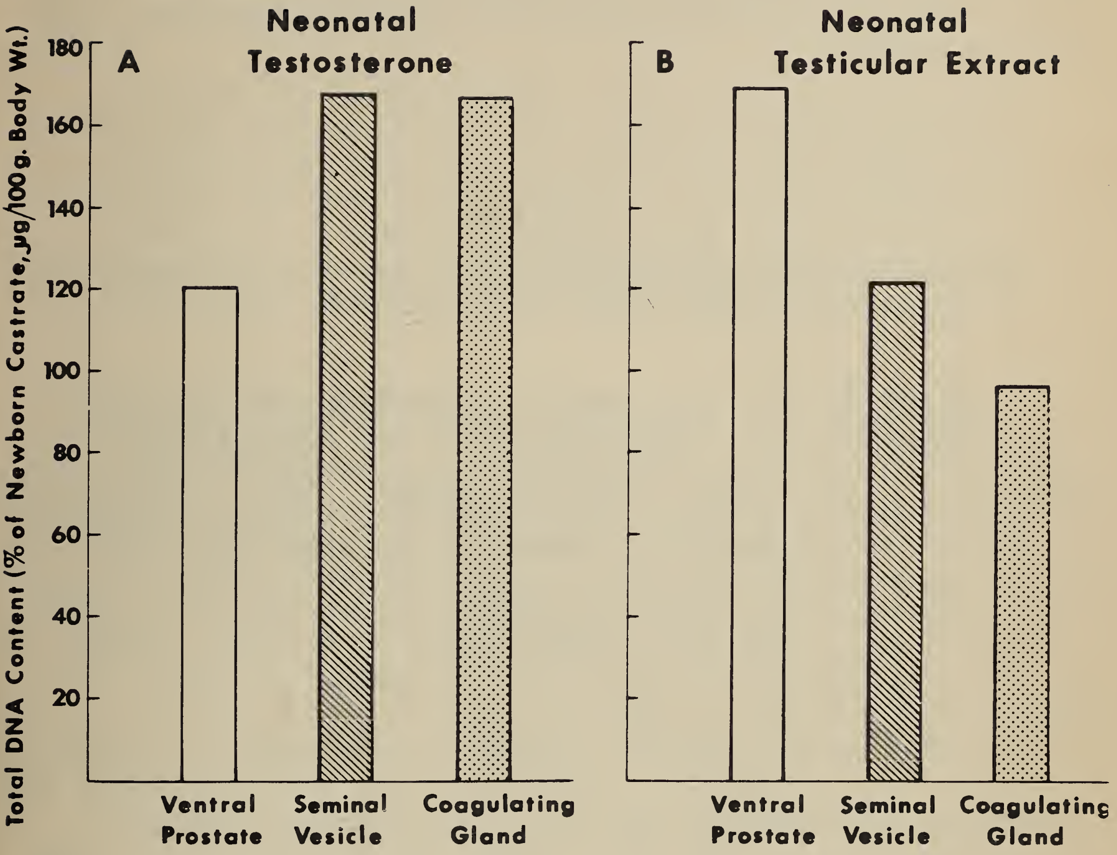

Figure 2. *The influence of the time of castration upon the adult responsiveness of sex accessory organs to testosterone proprionate. Rats were castrated at the indicated age. At age 130 days, a 10-day-course of testosterone proprionate, 2 mg/rat/day, s.c. was initiated. Animals were sacrificed after completion of the androgen administration. Responsiveness (DNA content) is expressed in comparison with the newborn castrate (100%).*

labelling of ventral prostate and seminal vesicles with ^{3}H-thymidine in the presence or the absence of neonatal testosterone. The accumulation and persistance of total DNA and total radioactivity incorporated into DNA of these glands was determined (Table III). Neonatal testosterone did enhance the incorporation of ^{3}H-thymidine into DNA, and also the accumulation of DNA, in both ventral prostate and seminal vesicle when assessed at age 10 and 20 days. At age of 70 days, however, the DNA content and the residual radioactivity in DNA of both glands were similar whether or not neonatal androgenization had taken place. And yet, only the seminal vesicles, but not the ventral prostate, from neonatally androgenized rats respond appropriately to the injection of exogenous testosterone. Once again, our ignorance of mechanism is emphasized.

TABLE III

EFFECTS OF NEONATAL TESTOSTERONE ON THE ACCUMULATION OF TOTAL DNA AND TOTAL RADIOACTIVITY FROM ^{3}H-THYMIDINE IN SEX ACCESSORY ORGANS*

Age (days)	Treatment of Newborn Castrate	Total DNA Content and ^{3}H-thymidine Labelled Into			
		Ventral Prostate		Seminal Vesicle	
		DNA Content (μg/gland)	Total ^{3}H-thymidine labelled into DNA (dpm/gland)	DNA Content (μg/gland)	Total ^{3}H-thymidine labelled into DNA (dpm/gland)
10	Oil	7.1 (6.1-8.1)	6700 (5200-8100)	12 (11-14)	11,400 (10900-11800)
	TP†	15 (12-18)	8390 (8200-8580)	29 (24-37)	13,200 (9800-18500)
70	Oil	41 (37-48)	3120 (2730-4130)	47 (46-48)	5590 (5360-5840)
	TP†	42 (41-44)	3700 (2770-4700)	49 (46-51)	5290 (4240-6860)

*Data in parentheses represent the range of these determinations. Three animals were studied in each of the four experimental groups. Animals were castrated on the day of birth. Tritiated thymidine (25 μC; 50 μg) was administered s.c. to all animals on day 2 and day 3.

†Treatment with testosterone constituted injection of testosterone proprionate, 500 μg/rat, in corn oil on day 2 and 3.

Liver

Sex hormones appear to regulate the levels of some hepatic steroid metabolizing enzymes. Although some of the effects occur at prepubertal or pubertal periods of development, others occur in the perinatal period. Indeed, Denef and De Moor[16, 17] found that neonatal testosterone can "imprint" away from the female pattern of cortisol metabolism toward the male type. Einarsson, Gustafsson and Sternberg[18] report that neonatal androgen is responsible for the "imprinting" of some microsomal steroid hydroxylating enzymes but has minimal effect on other enzymes. These investigators conclude that

> . . .microsomal steroid-metabolizing enzyme activities may be grouped into three classes with regard to the mechanisms regulating their activity: (a) enzymes with a basal activity level regulated by nongonadal factors but reversibly inducible by androgens; (b) enzymes irreversibly "imprinted" or "programmed" by androgens during the prepubertal period and reversibly stimulated by androgens postpubertally; and (c) enzymes primarily regulated by nongonadal factors and only slightly affected by androgens.

With respect to drug metabolism, a higher level of activity toward the metabolism of type I substrates (aminopyrine, ethylmorphine and hexobarbital) is found in male, as compared to female, rats.[19, 20] This difference, however, can be diminished by injecting androgenic hormones into female rats or estrogenic hormones into male rats.[20, 21] Levels of aminopyrine demethylase activity are low at birth but increase during development.[22] The differences in enzyme activity between male and female become apparent after the animal reaches sexual maturity.[22, 23] We have attempted to determine whether certain drug metabolizing enzymes, like specific steroid-metabolizing enzymes, are affected by neonatal testosterone. Physiologically, it is important to note that at birth rat testes contain large amounts of testosterone. This testosterone is depleted in the first five days of life with consequent rise in plasma concentration.[24]

Rats were castrated at varying times after birth, (newborn, 5, 10 and 20 days). Aminopyrine demethylase and aniline hydroxylase activities were then determined at later immaturity (6 weeks of age) and adulthood (21 weeks of age). The activity of aminopyrine demethylase in the castrated rats was reduced to approximately the female levels regardless of the time of castration. At 6 weeks of age, the enzyme activity will increase approximately two-fold to control male levels after 10 daily injections of testosterone (2 mg/rat/day, Figure 3). In the absence of testosterone stimulation, the lower levels of enzyme activity in the male castrates persist throughout life. At 21 weeks, however, no response of aminopyrine demethylase activity to the exogenous testosterone is observed. Rats castrated at the age of 20 days, however, do respond to the exogenous hormone at established maturity, (Figure 3). The underlying mechanisms of these findings are not understood. Experimental evidence indicates that these differences are explained neither by the dose of hormone nor the involvement of adrenal androgens.

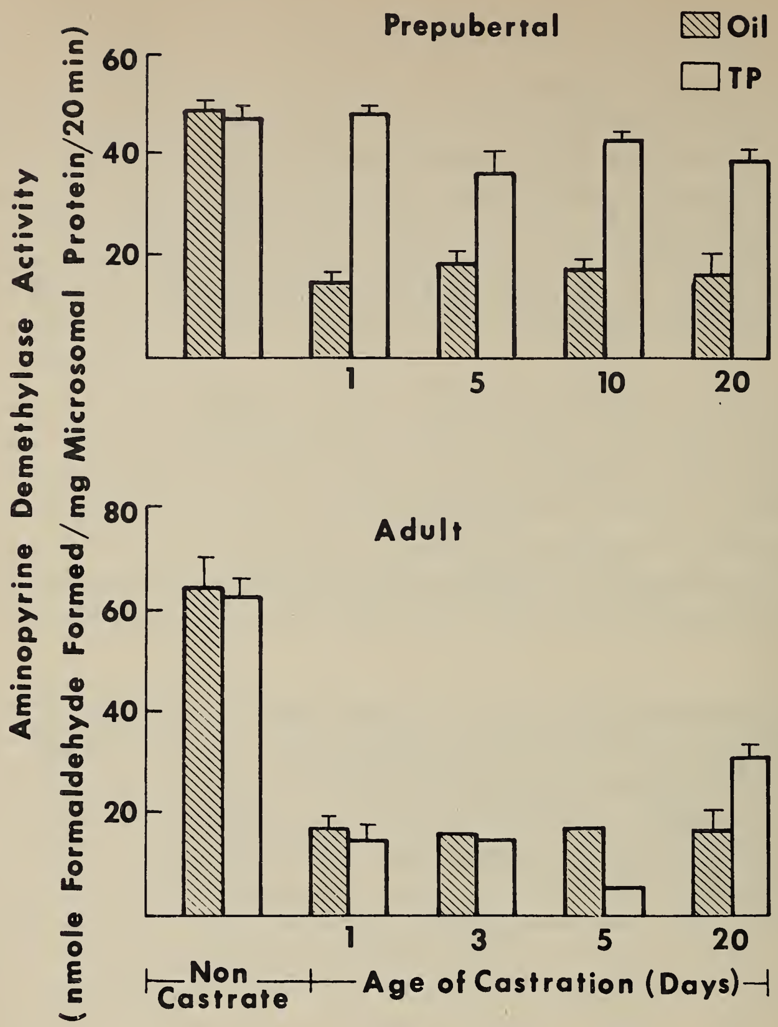

Figure 3. *Effects of castration at various ages on aminopyrine demethylase activity in rat liver microsomal fraction at age 50 days ("immature") and 150 days ("mature"), and the responsiveness to exogenous testosterone. Animals received injections of corn oil or corn oil with testosterone proprionate, 2 mg/rat/day for 10 days before sacrifice at age 50 or 150 days.*

In contrast to aminopyrine demethylase, no sex difference has been demonstrated for activities of enzymes that hydroxylate the type II substrates, e.g., aniline. Kato and Gillette[19] found that activity of aniline hydroxylase is not modified by the administration of androgens to immature castrated rats. We have extended these experiments to include rats castrated at birth, 5, 10 and 20 days in age. Figure 4 shows that in immature rats, basal levels of aniline hydroxylase were considerably lower in the newborn castrates than the non-castrated controls. The basal levels of activity increased as castration was delayed and reached the non-castrate basal levels when castration was performed at weaning. Androgen did stimulate the basal levels of aniline hydroxylase activity from groups castrated before weaning to the late castrate or non-castrate levels. It did not influence the levels of the enzyme in the rats castrated at weaning (Figure 4). Unlike the development of aminopyrine demethylase, aniline hydroxylase levels in all rats eventually developed the adult noncastrate pattern (Figure 4). At this period, androgen does not stimulate enzyme activity. Results of these studies add another dimension to the effects of neonatal androgen, namely, an influence on the ontogenetic, but not the mature, pattern of liver metabolism. It is not clear at this time to what extent adrenal androgen contributes to the development of aniline hydroxylase in liver.

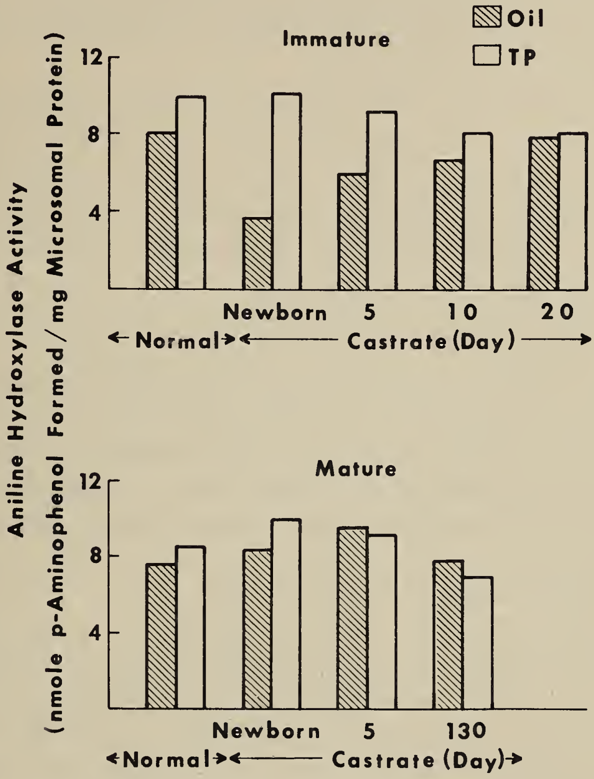

Figure 4. *Effects of castration at various ages on aniline hydroxylase activity in rat liver microsomal fraction at age 50 days ("immature") and 150 days ("mature"), and the responsiveness to exogenous testosterone. Animals received injections of corn oil or corn oil with testosterone proprionate, 2 mg/rat/day for 10 days before sacrifice at age 50 or 150 days.*

Determination of the total contents of cytochrome P-450 and microsomal protein reveal no difference between newborn, prepubertal or pubertal castrates and the normal controls. Testosterone administration to these rats did not alter the content of microsomal protein or cytochrome P-450. The data of enzymatic activities presented here merely represent the maximal velocity of these enzymes under these assay conditions. Kinetic and turnover studies analyses have not been performed. Gram *et al.*[25] showed that both the V_{max} and K_m values for aniline hydroxylase and ethylmorphine demethylase change during maturation through the neonatal and prepubertal periods.

PERINATAL CNS DEVELOPMENT

Although the capacities for gross movement and perceiving sensory phenomena are present in large part at birth, the perinatal human brain nevertheless is in a period of active development. This process continues into adulthood with development of coordinated movement, complex sensory integration, and the operations involved in intellectual tasks and behaviour. Documented physiological examples of the perinatal ontogenetic process in animals and man are abundant; one can cite, for example, changes in the electroencephalogram especially with respect to seizure patterns and threshold,[26] and the sleep-wakefulness cycle,[27] postulated to be due in part to maturation of thalamocortical systems.[28]

What factors are involved in the expression of these functional changes? And, especially in the context of this symposium, what *external* factors can influence the course of maturation, and how? There is obvious *inherent specificity* in the ontogeny of the brain. Indeed, at essentially every level, the "program" seems to arise predominantly from within the system. Nonetheless, interaction with external parameters, including drugs, can, indeed must, modify the program. Modification could occur at any level: the gene, gene activation, protein synthesis, protein function, intracellular multiprotein complex formation, organization of membranes, organelles, and other cellular components, and, finally, the organization of cells into functional multicellular systems. In the perinatal period, there seems to be increasing potential for external modification from one level to the next. The consequences, however, are increasingly likely to be subtle, and although potentially of great functional significance, increasingly difficult to detect, much less to quantitate.

Aspects of the Perinatal Ontogenetic Process

In man, the total brain weight increases from about 335 g at normal parturition to 1300 g in the adult,[29] a 300% increase. In the rat, which is born less mature than man, the increase is from 0.3 g to 1.9 g, or 530%.[30] This increase in total brain weight is due to a variety of factors, most of which are of greatest quantitative significance in the perinatal period.

Perinatal proliferation and differentiation of neurons. A large number of neurons are formed postnatally in both rat and man. These "microneurons" whose entire axonal-dendritic system lies within a single cortical region serve to modify input to and output of large neurons that convey information from one to another region of the CNS. This process has been studied in most detail in the cerebellar cortex. The only output from the cerebellum is via Purkinje cell axons. Thus all input to the cerebellum eventually reaches the Purkinje cells. However, the incoming information is integrated and modulated by a complex, highly regular spatial arrangement of excitatory and inhibitory microneurons[31] within the cerebellar cortex. These microneurons, as well as glia, are derived from the external germinal zone, a layer of several cells thickness that covers the developing cerebellum at birth. Germinal cells proliferate and, with differentiation, generate the microneurons which migrate to form two of the layers of the cerebellar cortex. These processes are complete at about day 20 and 30, respectively in rat[32] but not until age one or two years in man.

Increase in neuronal dendritic field. After migration, neurons begin a period of maturation during which the dendritic field and hence potential synaptic connections increase markedly. This is reflected by an increase in the ratio of total volume/ cell number. The ratio rises dramatically in the cerebral cortex of man from birth to age four years.[29]

Synaptogenesis. The organization of cells into multicellular systems with highly specific and characteristic spatial patterns in the CNS occurs most rapidly in late gestation and early postnatal life. The patterns of cells in the CNS seem to be specified functionally. These spatial patterns are dependent on the temporal appearance of cell types and the specificity and relative strength of interactions between cells. Relative strength implies modifiable connections or neuronal plasticity.

Increase in glial cell number. There is a considerable increase in the glia/neuron index in the early postnatal period, a trend that continues slowly even into adult life.[33]

Myelinization. Myelinization occurs at different times in different fiber tracts; net formation can persist into adulthood in man.[34] The formation of myelin proceeds most vigorously in many fiber tracts in the perinatal period.

Metabolism. There are indications that the perinatal brain may utilize ketone bodies as fuel to a much greater extent than does that of the adult.[35]

Biochemical changes during this period of rapid CNS development do correlate with anatomical and physiological changes. Thus DNA content and synthesis are correlates of total cell number and rates of neuroblast and glial proliferation. The DNA content of the CNS in man is maximal at about 8 months of age with the highest postnatal rates of change seen in the cerebrum and cerebellum. DNA content in the cerebellum of the rat increases 580% between postnatal days 2 and 14, the period corresponding to rapid proliferation of neuronal and glial precursor cells in the external germinal zone.[36] Rates of RNA and total protein synthesis are crude correlates of differentiation and morphological maturation.[37] Similarly gangliosides may offer a crude measure of synaptogenesis.[38, 39]

Changes in levels of acetylcholine, catecholamine, 5-hydroxytryptamine, and other transmitter candidates, as well as developmental changes in the enzymes related to their biosynthesis and degradation, have been used frequently in the study of appropriate fiber tracts and neuronal populations. Myelin basic protein, and lipids such as sulfatides, serve as specific markers for the process of myelinization. Acidic S-100 protein appears to be specific for glial cells and has been used as an index of the glial maturation. Recent use has been made of an astrocyte-specific protein, different from S-100, to study astrocyte differentiation in the cerebellum and pyramidal tracts of the newborn rat.[40] Our laboratory has found D-amino acid oxidase, a flavo-protein of as yet unknown function, to be highly localized in certain cell types in the cerebellum[41] with a dramatic developmental pattern as determined biochemically and histochemically.

The purpose of enumerating these aspects of perinatal brain maturation is not to be encyclopedic, but rather to stress the activity of ontogenetic processes at this period of life. It is obvious that little is understood mechanistically about these processes, much less to what degree they are susceptible to permanent modification or injury. Indeed, in view of the subtle and complex character of perinatal imprinting by neonatal androgen, it is disturbing to acknowledge the inadequacies of the methods now available for assessment of significant residual modification of the perinatal brain.

Agents Associated with Permanent Alteration of the Perinatal CNS.

X-Irradiation. The effects on CNS development of low level irradiation in the early postnatal period have been studied extensively by Altman and others. Dramatic differences exist with respect to radiosensitivity, with proliferating migratory cell precursors and postmitotic migratory cells being particularly radiosensitive. Cells whose soma has already reached its final destination (e.g., Purkinje cells in cerebellum) are much less sensitive.[42] While radiosensitivity is not well understood on the molecular level, the cell does not have to be synthesizing DNA to be sensitive; protein destruction with lack of sufficient repair capacity has been implicated.[43] The effects of low level irradiation on the developing rat cerebellum are a function of the time of irradiation and number of exposures. Thus, Altman and Anderson[44-47] have been able to selectively destroy different cell populations originating from the cerebellar external germinal zone (EGZ) by irradiating the cerebellum at times when the cells to be eliminated are being formed and migrating. Complete elimination of the EGZ results in a cerebellum completely devoid of microneurons. Single doses which eliminate only a portion of the external germinal zone give varying results due to the ability of the EGZ to replenish precursor cells. The capacity for "regeneration" in perinatal brain remains scarcely explored and of potentially great functional significance. When rats are irradiated between postnatal days one and ten, there is almost complete recovery as assessed by cerebellar size and cell numbers; however, irradiation after day 10 provokes permanent alterations due to the limited ability of the EGZ to regenerate in the time between injury and programmed cessation of proliferation (about 20 days postnatally in the rat). It should be noted that behavioural abnormalities have been observed in rats irradiated between 1 and 3 days postnatally even though certain parameters such as total cerebellar weight were unaffected.[48] This suggests that subtle, yet functionally significant, changes are likely to have occurred, but which are undetected by current methodology. The need for more specific biochemical indicators of abnormalities in development as well as appropriate physiological and neurological tools to detect subtle functional abnormalities is again emphasized.

Methylazoxy methanol (MAM). MAM selectively destroys actively dividing cells probably by methylation of the 7-position of guanine in nucleotides.[49] Treatment of rats with MAM results in destruction of the EGZ in dose-dependent fashion. When administered between postnatal days one to four, in a dose that does not completely destroy the EGZ, initial destruction of germinal cells is observed followed by recovery so that the cerebellum is essentially "normal" as assessed histologically,[50] as well as by the parameters of total cell number (DNA) and size (protein, total cerebellar weight).[51]

Of particular interest is the fact that cycad seeds, which contain cycasin, the β-glucoside of MAM, constitute a significant portion of the diet of native populations in certain Pacific islands, especially Guam.[52] Neurological disorders occur with an extraordinarily high frequency among these populations.[53-55] When adult mice, pigs, and horses were fed a diet containing cycad seeds no pathological changes were detected.[56] However, cycasin, when administered to mice and hamsters during the first few days of postnatal life, caused significant neurological and pathological abnormalities including moderate to severe reduction of microneurons in the cerebellum.[57-59] The abnormalities seen in man and those seen in experimental animals are different enough, however, to necessitate caution in concluding that cycasin is the cause of the human disorders.

Histological abnormalities of the cerebellum have also been observed in hamsters treated in the neonatal period with the nucleoside analogue, cytosine arabinoside[60] and the antineoplastic cytotoxin, cyclosphosphamide.[61] Studies in our laboratory with cyclophosphamide administered to Wistar rats at ages 2, 7 and 13 days produced severe growth retardation, a reduction in total brain and total cerebellar weights, and a reduction in total brain and total cerebellar DNA content in the adult animals. However, DNA, RNA, protein and cholinesterase concentrations per unit weight of fresh adult cerebellum were normal in the treated groups. In addition, temporary changes in gross cerebellar cortical histology, observed shortly after treatment, were not observed in the adult animals.

6-Hydroxydopamine. 6-Hydroxydopamine has been shown to destroy adrenergic terminals (norepinephrine and dopaminergic) in adult animals in both the peripheral and central nervous systems.[62] When the compound is administered to the newborn animal, the entire peripheral neuron is destroyed, while only terminals are destroyed when adult animals are treated, thus permitting potential regeneration in the adult only. However, the effects on the newborn CNS in this regard are still unclear. 6-Hydroxydopamine is more toxic to the CNS in the perinatal period; this is due, at least in part, to differences in the blood brain barrier.[63] The effects of 6-hydroxydopamine on CNS neurons is complicated by differential sensitivity. Noradrenergic neurons are more sensitive than dopaminergic,[62] but, in addition, there may be regional differences in sensitivity among different populations of the same class,[64] Other derivatives such as 6-aminodopamine have been shown to have similar effects on adrenergic neurons[65, 66] as have polyhydroxytryptamines on serotoninergic neurons.[67] These compounds are thought to exert their action by accumulating in nerve terminals due to their structural resemblance to norepinephrine and dopamine or 5-hydroxytryptamine and then cause structural damage possibly by covalent binding of auto-oxidation products.[62, 68]

Recent studies have shown that perinatal treatment of rats with psychotropic drugs such as methamphetamine and chlorpromazine leads to permanent regional alterations in biogenic amine concentrations in the brain of the adult animal.[69, 70] Although the mechanism is, for the present, obscure, the functional implications are great.

Hormones. Hormones can have at least two types of actions on the perinatal CNS. One is a highly specific "imprinting", and the other a relatively non-specific "anabolic" effect. For example, the presence of thyroid is necessary for normal CNS development.[71] Hyperthyroidism results in an early disappearance of the EGZ in developing cerebellum as well as abnormal early cell differentiation. Hypothyroidism causes prolonged germinal cell proliferation and retarded differentiation. In both cases, there is decreased brain and cerebellar weight and quantitative abnormalities in the microneuron and glia populations, as well as a terminal deficit in total synaptic profiles in the cerebellar cortex.[9] It is of well known clinical importance that hypothyroidism be diagnosed and treated early, especially in the perinate.

The influences of perinatal nutrition and experience in the development of the CNS are subjects of current interest.[73, 74] The concept of plasticity in neuronal connections, probably significant only in microneurons postnatally, implies the potential for modification by environmental input and use-disuse. For example, electrophysiological changes in the visual cortex occur in cats deprived of light from birth, but do not occur if the deprivation starts later than 6 weeks after birth.[75-77] This would be consistent with the concept of a critical period of neuronal synaptic plasticity, after which many may become specified[78] and permanent.

In summary, a case for the subtle and complex character of perinatal residual and/or delayed toxicity has been developed with use of neonatal "imprinting" by androgens. Certain active perinatal ontogenetic events occurring in the perinatal CNS are reviewed and a brief tabulation of those agents known to be associated with permanent changes in the perinatal brain is presented. It is our goal to stimulate further concern and investigation into these questions.

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The Use, Efficacy, and Toxicity of Psychoactive Drugs in Hospitalized Medical Patients:

A Report From
The Boston Collaborative Drug Surveillance Program

Samuel Shapiro, M.D. and Hershel Jick, M.D.

ABSTRACT Almost half of a medical inpatient population received psychoactive drugs. Efficacy was rated by physicians as satisfactory in about 75 per cent of the cases. Drowsiness was the most commonly reported side effect. Extrapyramidal symptoms were uncommon and seen with most frequency in patients receiving trifluoperazine.

Most clinical investigators are aware that medical patients in hospital are exposed to a wide variety of drugs. Nevertheless, many will probably be surprised to learn of the extent to which psychoactive drugs are used by patients. Psychoactive agents here refer to drugs used to treat behavioral symptoms which range from anxiety to grossly psychotic behavior.

We would like to describe briefly the usage patterns of these drugs in medical inpatients; to provide some quantitative data on their efficacy and toxicity; and also to discuss factors which appear to influence efficacy and toxicity.

The study from which these data are derived is the Boston Collaborative Drug Surveillance Program. This study has been ongoing since July 1966. Some fifteen hospitals in five countries have participated at one time or another (Table I). Since the methods of this study have been repeatedly reported, we shall only mention them briefly.[1] Nurse monitors are stationed in medical wards where they collect information on consecutively admitted patients. The information includes the usual descriptive characteristics of each patient, including the diagnosis, and details of all drugs administered. When a treatment is started, the nurse monitor interviews the attending physician to determine the indication; when a drug is stopped, she asks the doctor to judge its efficacy. At the same time she asks whether or not there had been an adverse reaction.

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TABLE I

MONITORED HOSPITALS

		Total Patients	Total Admissions
Lemuel Shattuck Hospital	(Boston)	2,651	3,785
Boston City Hospital	(Boston)	971	1,180
Westminster Hospital	(Canada)	98	103
University Hospital	(North Carolina)	77	77
Veterans Admin. Hospital	(Boston)	2,680	3,063
Peter Bent Brigham Hospital	(Boston)	2,890	3,414
Mass. General Hospital	(Boston)	1,006	1,099
St. Joseph's Hospital	(Canada)	976	1,040
Roger Williams Hospital	(Rhode Island)	1,834	1,947
Hadassah Hospital	(Israel)	1,546	1,700
Auckland Hospital	(New Zealand)	454	480
University Hospital	(Boston)	42	42
Beilinson Hospital	(Israel)	–	–
Western Infirmary, Glasgow	(Scotland)	–	–
Asaf Harofe Hospital	(Israel)	–	–
	TOTAL	15,225	17,930

Table II shows the usage pattern of the eleven most commonly prescribed psychoactive drugs. Over one-third of all patients monitored received diazepam or chlordiazepoxide, usually for anxiety. An additional 9 per cent received phenobarbital primarily for anxiety. Although there was some overlap – *i.e.*, some individuals received more than one of these drugs – it is apparent that a very large percentage of patients was exposed to at least one drug. One out of 25 patients received chlorpromazine. The remainder of the drugs were prescribed less frequently.

TABLE II

USE OF PSYCHOACTIVE AGENTS IN MEDICAL POPULATIONS

Drug	# Exposed	% of Total
Diazepam	3238	21
Chlordiazepoxide	2188	14
Phenobarbital	1349	9
Chlorpromazine	566	4
Meprobamate	153	1
Hydroxazine	137	1
Thioridazine	116	<1
Perphenazine	91	<1
Promazine	90	<1
Imipramine	81	<1
Trifluperazine	75	<1

Table III presents the ten most common indications for drug therapy. Anxiety is the eighth most common and accounts for fully 4 per cent of all drug orders.

TABLE III

TEN MOST COMMON INDICATIONS FOR DRUG THERAPY

Indication	Total Exposures %
Constipation	10.0
Insomnia	9.3
Pain	8.7
Infection	7.1
Congestive Heart Failure	4.8
Dehydration	4.4
Vitamin depletion	4.2
Anxiety	4.0
Electrolyte depletion	3.9
Bronchospasm	3.6

Table IV presents the overall physician efficacy ratings for the four most commonly used psychoactive agents. The drugs are not strictly comparable since they may have been given to different types of patients for a variety of symptoms and at varying dosages. This is, of course, particularly true of chlorpromazine. Nevertheless, one gets some reasonable estimate of the physician's evaluation of efficacy from these data. The results for each drug are closely similar and indicate that a satisfactory result is thought to have been obtained in about three-fourths of the patients treated. This is probably encouraging and the results compare favorably with those obtained with other drugs used for relief of a variety of symptoms such as insomnia or pain. It should be emphasized that we do not have any indication of what the "success" rate would have been, had a placebo been given. Thus, these data do not provide an estimate of efficacy directly attributable to the pharmacologic activity of these drugs. They simply indicate that, for whatever reason, these agents are thought to satisfactorily relieve the symptoms they were prescribed for in a substantial majority of patients. One could also draw a tentative conclusion that diazepam, chlordiazepoxide and phenobarbital are reasonably comparable in efficacy in this setting.

TABLE IV

EFFICACY OF PSYCHOACTIVE DRUGS

Drug	Satisfactory %	Unsatisfactory %	Don't Know %
Diazepam	77	8	15
Chlordiazepoxide	80	7	13
Phenobarbital	79	9	12
Chlorpromazine	74	12	14

Adverse reaction rates for the drugs discussed are presented in Table V. The rates for the three most commonly used drugs are reasonably similar. What differences there are may be explained by certain differences in the type of patient receiving the drugs. Approximately three-fourths of the reactions attributed to these drugs was CNS depression described as "drowsiness." While this was usually of relatively minor clinical relevance, it did induce the physician to discontinue treatment in over 80 percent of cases, and severe CNS depression leading to coma and death has occured with these drugs.[2]

TABLE V

ADVERSE REACTION RATES TO PSYCHOACTIVE AGENTS

Drug	Exposed N	With Adverse Reaction N	%
Diazepam	3238	238	7.4
Chlordiazepoxide	2188	229	10.5
Phenobarbital	1349	122	9.0
Chlorpromazine	566	71	12.5
Meprobamate	153	4	2.6
Hydroxazine	137	4	2.9
Thioridazine	116	18	15.5
Perphenazine	91	5	5.5
Promazine	90	8	8.9
Imipramine	81	9	11.1
Trifluperazine	75	13	17.3

We have found a number of factors which appear to influence the frequency of CNS depression attributed to some of the psychoactive agents.[3] Table VI reviews our data on the relationship of drowsiness attributed to diazepam, chlordiazepoxide and phenobarbital to smoking and age. These data show a strong independent correlation between diazepam and Librium-induced drowsiness and smoking: *i.e.*, smokers developed drowsiness substantially less often than non-smokers; and to age: *i.e.*, older patients developed drowsiness more often than younger patients. These findings were independent of dose. Phenobarbital-attributed drowsiness did not show these relationships.

TABLE VI

DROWSINESS IN RECIPIENTS OF BENZODIAZEPINES AND PHENOBARBITAL IN RELATION TO AGE

	Benzodiazepines		Phenobarbital	
Age	No. of Patients	% With Drowsiness	No. of Patients	% With Drowsiness
Nonsmokers:				
⩽40	134	5.2	24	4.2
41-50	136	5.9	21	9.5
51-60	193	8.8	22	4.5
61-70	211	7.6	17	0.0
>70	186	12.9	17	11.8
Smokers:				
⩽40	294	4.1	45	11.1
41-50	317	3.5	47	2.1
51-60	309	6.8	44	6.8
61-70	160	10.0	19	10.5
>70	62	4.8	16	12.5
Totals:				
⩽40	428	4.4	69	8.7
41-50	453	4.2	68	4.4
51-60	502	7.6	66	6.1
61-70	371	8.6	36	5.6
>70	248	10.9	33	12.1

We would like to describe briefly our experience with the incidence of drug-induced extrapyramidal symptoms (EPS) attributed to psychoactive agents.[4] Three classes of psychoactive drugs have been implicated in the induction of EPS: phenothiazines, tricyclic antidepressants and butyrophenones.

A total of 2,049 patients received one or more of these drugs. This includes over 1,100 patients who received prochlorperazine prescribed primarily for nausea. Table VII summarizes the data on this subject. Out of a total of 2,049 patients receiving one or another of these drugs, singly or in combination, 18 (0.9%) developed EPS. The drug which caused EPS most often was trifluoperazine: seven of 63 patients exposed developed this problem.

TABLE VII*

EXTRAPYRAMIDAL SYMPTOMS ACCORDING TO CLASS OF DRUG, TYPE OF PHENOTHIAZINE, AND COMBINATIONS OF PSYCHIATRIC DRUGS AND PREDNISONE

Drugs Taken Prior to Symptoms	No. Exposed	Extrapyramidal Symptoms	
		No.	%
Phenothiazine	1,782	14	0.8
Tricyclic antidepressant	173	2	1.2
Butyrophenone	4	1	25.0
Phenothiazine and tricyclic antidepressant	90	1	1.2
None of the above	9,830	0	0
Total	11,879	18	0.15
Prochlorperazine	1,001	5	0.5
Chlorpromazine	321	1	0.3
Prochlorperazine and chlorpromazine	72	1	1.4
Trifluoperazine hydrochloride	33	5	15.2
Trifluoperazine hydrochloride and perchlorperazine or chlorpromazine	30	2	6.7
Total	1,457	14	1.0
Trifluoperazine hydrochloride	55	4	7.3
Trifluoperazine hydrochloride and prednisone	8	3†	37.5
Other psychiatric drugs	1,756	9	0.5
Other psychiatric drugs and prednisone	230	2	0.9

*Taken from ref. 4

†Female patients with systemic lupus erythematosus.

In summary, the usage patterns of psychoactive drugs in a medical inpatient population have been described. These drugs are received by almost half of the admitted patients. Efficacy is thought to be satisfactory in about three-quarters of recipients. Drowsiness is the most commonly reported side effect and for some drugs this side effect is correlated with smoking and age. Extrapyramidal symptoms attributed to psychoactive drugs are relatively uncommon but have been seen with substantial frequency in patients receiving trifluoperazine.

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Epidemiology and Therapy of Suicidal Drug Intoxication

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ABSTRACT In a prospective study of 169 patients with acute poisoning admitted to the Medical Intensive Care Unit of the Montreal General Hospital in 1972 and the first half of 1973, benzodiazepines and non-barbiturate hypnotics were most frequently encountered. A progressive decrease in the incidence of phenothiazines, tricyclic antidepressants and street drugs has been observed but this has been offset by an increase in the use of benzodiazepines and salicylates. The concomitant ingestion of alcohol was extremely high. Thirty-seven per cent of the patients had taken an overdose of a drug prescribed for them by their psychiatrist or other physician. A previous suicide attempt was recorded in 50% of patients and less than one third of this group were under present psychiatric care. Unconsciousness, respiratory depression and mild hypotension were the most frequent clinical abnormalities. Treatment was supportive and included the instillation of activated charcoal after gastric lavage as a means of decreasing drug absorption. The need to enhance drug clearance by either forced diuresis or dialysis was infrequent. There were eight deaths. Respiratory complications occurred in about one fourth of the patients. Average duration of coma was short. Only seven surviving patients remained unconscious for more than 24 hours. Toxicologic analysis influenced treatment in 22 patients, 14 of which were salicylate intoxication.

It is recommended that more attention be paid to recognizing patients whose behavioural pattern includes repeated suicide attempts, that alternatives be found for barbiturate and non-barbiturate hypnotics, and that management of severe drug poisoning in the adult should be conservative.

We have recently reported our experience in the treatment of acute drug poisoning in adults[1] at the Montreal General Hospital for the period of 1972. That report has helped fill the gap of reported Canadian experience in adult drug overdose compared to the vast information available on the pediatric group through Poison Control Centres. This paper expands that experience into 1973.

METHODS

Details of 169 patients admitted with acute poisoning during 1972, and in the first 6 months of 1973 were recorded prospectively and transferred to punch cards. No

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change was made in the manner in which these patients have been treated for the last five years in this hospital. All patients were admitted to the Medical Intensive Care Unit, where their care was supervised in consultation by the Division of Clinical Pharmacology and Toxicology. This group of patients made up 18% of admissions to the Intensive Care Unit.

The established method of grading coma[2] was not used in this study because many of our patients did not fit the criteria for any grade. Instead, patients were considered in three major groups: those who were in coma and showed no response to pain, those who were in coma but showed response to pain, and those who were drowsy, semi-conscious or alert. Patients were considered to have regained consciousness when they could respond to simple commands.

Establishment of the diagnosis of drug overdose as the cause of coma was usually made from the history given by the patient or those who accompanied him to hospital. In the majority of cases, blood, urine and stomach contents were collected for toxicologic analysis but actual processing of the samples was usually carried out at a later time unless emergency analysis was deemed necessary by the treating physician.

Data for the one-and-a-half-year period were divided into three 6-month portions. Grouped data for the entire one and one half years indicate no major difference between these periods.

RESULTS

Pattern of Drug Overdose

The decrease in incidence of barbiturates and salicylates as putative drugs reported by others[3-6] in reviews in England was more marked in our experience. Figure 1 shows that the benzodiazepines (most often diazepam) were the commonest group of drugs taken by patients either alone or in combination. The non-barbiturate hypnotics ran a close second with Mandrax® (methaqualone with diphenhydramine) and Noludar® (methyprylon) making up the majority. Methaqualone alone, chloral hydrate, glutethimide, and ethchlorvynol made up the remainder. Use of propoxyphene was rare in contrast with the experience of others.[7] The preponderance of benzodiazepines and non-barbiturate hypnotics reflects the prescribing habits of the physicians in this area. The remaining groups of drugs were each implicated to a fairly similar percentage. When the study period was divided into three 6-month intervals, there were fairly similar numbers of patients during each period. We observed a progressive decline in the incidence of the phenothiazines, tricyclic antidepressants and street drugs (Table I). It would appear that the prescribing habits of our psychiatrists are directing away from inappropriate use of the psychotropics. However, it is quite apparent from the unchanged overall incidence of overdose that the patient will not be deterred in his attempt. He will merely switch to another more readily available drug as seen by the increased incidence of use of benzodiazepines and salicylates. The decrease in the incidence of street drug use from our recorded high of 20 percent in 1971 is encouraging and hopefully represents the waning of a fad. The decline in LSD and amphetamine abuse may now be supplemented by marijuana. We are not so naive as to completely believe that this decrease

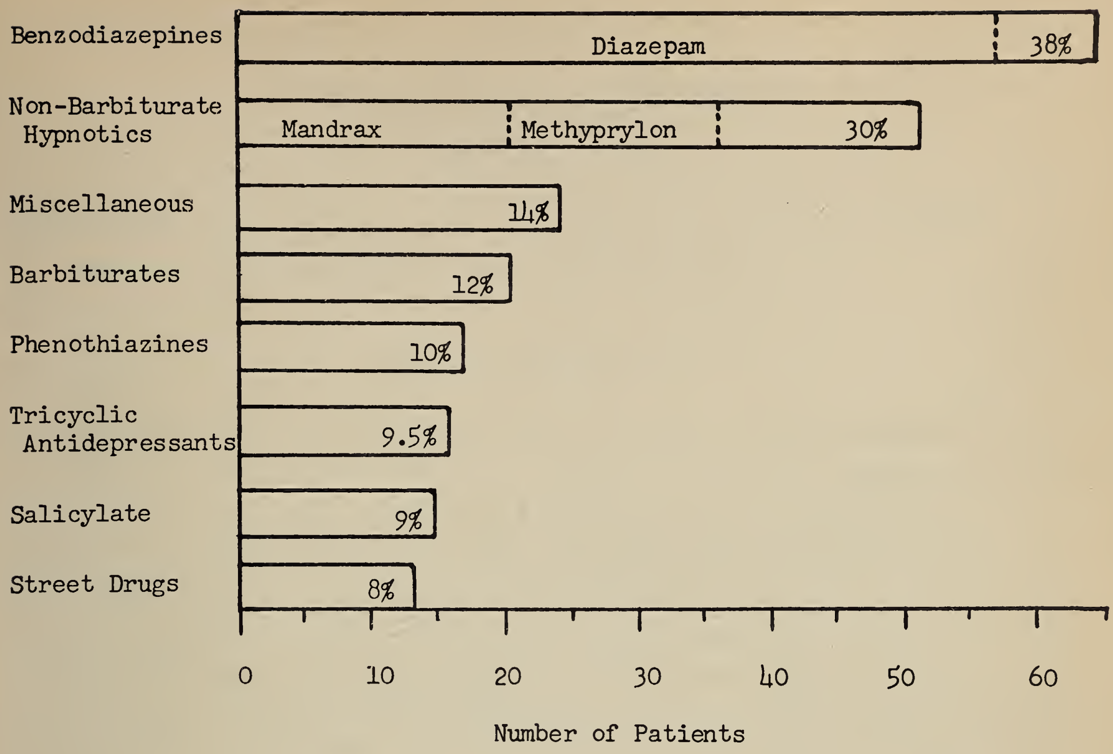

Figure 1. *Drugs Taken in Overdose by Patients over one-and a half years.*

in incidence is real since it may also represent increasing experience and sophistication in the use of these agents or that these patients are utilizing other agencies for treatment of such problems.

TABLE I

TRENDS IN DRUG USE FOR OVERDOSE PATIENTS ADMITTED

Drug	Jan-June/72 N = 59 %	July-Dec/72 N = 49 %	Jan-June/73 N = 61 %
Phenothiazines	24	17	10
Tricyclics	24	19	7
Street Drugs	13	8	5
Benzodiazepines	33	29	49
Salicylates	10	12	24

TABLE II

SOURCE OF DRUGS TAKEN BY 169 PATIENTS ADMITTED WITH ACUTE POISONING
(Jan 72 – July 73)

Source	% of Patients
Psychiatrist	29
Physician	8
Friends	12
Unknown	51
Drug with Alcohol	24
More Than 1 Drug	88

Although there was a decrease in the use of psychotropic drugs over the study period, the percentage of patients who acquired their drug through a psychiatrist remained constant at around 29 percent (Table II). Physicians accounted for only 8% of source while friends and relatives, etc., were the source in 12% of patients and the source was unknown in over half of our group. Drug was taken with alcohol in 24% of the group; however, these were based on history alone and were not confirmed by routine assay for ethanol in the blood. A recent British study[8] in which routine assay for ethanol was carried out in all patients presenting with self-poisoning, showed over 75% of patients had blood alcohols greater than 100 mg/100 ml. The implications of these findings are great, and extensive clinical research is warranted since alcohol can have at least additive CNS depressant effects and in addition, may decrease the rate of hepatic metabolism by the liver.[9] Although ethanol may have a direct inhibitory effect on the hepatic enzymatic degradation of common drugs, it is also possible that clearance of the drug is diminished by decrease in delivery of the drug to the liver, *i.e.* decreased hepatic blood flow or by decreased hepatic uptake of the drug. These latter possibilities are unknown in man and should be studied since the use of alcohol is so ubiquitous in our society. Since ethanol is justifiably considered a drug, then more than one drug was implicated in close to 90% of our patients.

TABLE III

CHARACTERISTICS OF 169 PATIENTS ADMITTED WITH ACUTE POISONING

	% of Patients
Men	39
Women	62
Previous Suicide	50
Previous Drug Suicide	43
Past Psychiatric Care	20
Present Psychiatric Care	28

Mean Age (yrs. ± S.D.) = 35 ± 12

Patients were young, with an average age of 35 years (Table III). The proportion of men in our patients was higher than in other studies.[3, 4] At least half of the patients had made a previous suicide attempt by either drug or non-drug methods. A very disturbing 43% of patients had a previous history of suicide attempt with drugs. While 28% of all patients were under present psychiatric care, only 32% of those patients with a previous history of suicide attempt were under psychiatric care. This latter figure may account for the "repeater" incidence and may indicate an area where greater preventive psychiatric care could be directed.

MEDICAL MANAGEMENT

The principles of treatment have been those of intensive supportive care as outlined by Matthew and Lawson.[2] In addition to support of ventilation, aggressive volume replacement usually with normal saline was used to treat the absolute and relative hypovolemia as demonstrated by Shubin and Weil[10] to occur in severe drug overdose. The mechanism of the relative hypovolemia is unknown but studies with diazepam[11] and thiopental[12] suggest increased venous capacitance. Preliminary studies of venous compliance in our patients seem to verify this concept. A secondary benefit of improving cardiovascular status could be improved hepatic blood flow. Hepatic blood flow (*i.e.* delivery of drug to the liver) appears to be the major determinant in the clearance of some drugs.[13] Its importance in drug overdose should be studied.

TABLE IV

TREATMENT OF 169 PATIENTS ADMITTED
WITH ACUTE POISONING

Treatment	Number of Patients Coma Without Pain Response N = 42	Coma With Pain Response N = 60	Semi coma or Conscious N = 67	% of Total
Lavage	41	51	43	80
Charcoal	40	49	43	78
Ventilation	35	39	11	50
Resuscitation	3	3	1	4
Catecholamines	8	3	1	7
Diuresis	6	4	11	12
Dialysis	1	1	2	2

Fifty percent of the patients admitted to hospital required endotracheal intubation and assisted ventilation because of respiratory depression (Table IV). Cardiac arrest occurred infrequently and was usually confined to moribund patients presenting in Emergency. The use of catecholamines to support tissue perfusion was infrequent since we do not believe the etiology of the hypotension to be low peripheral vascular resistance. Isoproteronol was used primarily for bradyarrhythmias. Norepinephrine was occasionally used as an emergency measure to maintain an adequate perfusion pressure until sufficient volume replacement had been achieved. Forced osmotic diuresis was used in 12% of cases. Of five patients dialyzed, three had taken methanol and were treated with combined peritoneal dialysis and hemodialysis. Two of these patients survived. One patient received peritoneal dialysis in the treatment of acute renal failure secondary to ethylene glycol poisoning and one patient with very severe salicylate intoxication was successfully treated with a combination of peritoneal and hemodialysis. In our experience, with the possible exception of methanol and severe salicylate overdose with complex acid-base disturbances, dialysis does not affect the outcome of our patients as assessed by survival or complications. Our Nephrology consultants concur with this approach. We believe one of the reasons for our infrequent need for dialysis is the routine use of activated charcoal after gastric lavage.

It has been our practice for the last five years to instil 100 gms of activated charcoal, as a slurry, into the stomach after gastric lavage, with the anticipation that drug remaining in the small bowel would be adsorbed onto this non-specific binding substance.[10] The administration of activated charcoal has been shown to be extremely efficient in reducing the intestinal absorption of therapeutic doses of some drugs in man.[14] Although we have reason to believe that routine administration of activated charcoal influences the mortality and morbidity of our patients, definite proof remains to be demonstrated.

IMMEDIATE RESULTS OF TREATMENT

Although death from drug poisoning was unusual, the complications of the condition and possibly of the treatment were common in our patients (Table V). There was a 27% incidence of respiratory complications, the greatest number occurring in those patients in coma without response to pain. The majority of these were considered to be aspiration pneumonia either at the time of admission or during the treatment period. The clinical and radiologic signs of aspiration pneumonia usually resolved rapidly, and rarely required specific therapy. Intubation of the mainstem bronchus occurred occasionally during transport of the patient from Emergency. Although we are concerned with this relatively high incidence of respiratory complications, we have found some consolation in a study of patients with drug overdose treated in the UCLA Respiratory Care Unit, which shows twice our incidence of aspiration pneumonia.[15] Contrary to the myth, bullous skin lesions were not confined to barbiturate overdose. Other complications were rare.

TABLE V

DEATHS AND COMPLICATIONS IN 169 PATIENTS ADMITTED WITH ACUTE POISONING

	Number of Patients			
	Coma Without Pain Response	Coma With Pain Response	Semicoma or Conscious	% of Total
Deaths	6	2	0	4.7
Respiratory Complications	20	16	10	27
Convulsions	3	1	3	2
Skin Lesions	4	2	1	4
Withdrawal	1	1	1	2

Eight patients died. This represents 4.7% of the total number of patients admitted or 8% of the patients in severe coma. Either of these figures is appreciably less than the 29% mortality reported by Shubin and Weil for a seemingly similar group of patients treated in a similar Intensive Care Unit.[16] If our death rate is extrapolated to the total population presenting in Emergency with the diagnosis of drug overdose (ratio of 4 in 1 admitted) then our death rate is about 1%. Four of the eight patients who died were admitted to the Emergency Department in cardiorespiratory arrest. All responded transiently to resuscitative measures but none regained pupillary reflexes, deep tendon reflexes or activity on electroencephalograph. Drugs invoked were phenmetrazine with cocaine, secobarbital with amobarbital, trimipramine, and cyanide. Our patient with ethylene glycol poisoning was transferred to the hospital anuric with multifocal cortical seizures and severe metabolic acidosis (arterial pH 6.89). Treatment was terminated after 15 days of anuria and no evidence of return of neurologic function. One 78 year old male with terminal carcinoma of the head of the pancreas took an overdose of secobarbital, recovered consciousness, but died shortly thereafter with signs of increasing obstructive jaundice. His death was not considered drug related. One 68 year old female was admitted with salicylate overdose (serum salicylate concentration, 58 mg per 100 ml), a left hemiplegia of recent onset, respiratory alkalosis, metabolic acidosis, and congestive cardiac failure. She died eight hours after admission, of respiratory arrest. One 67 year old male presented 72 hours after accidental ingestion of a large quantity of methanol. His initial stuporous, semi-blind state rapidly deteriorated to a terminal cardiac arrest in spite of aggressive measures to correct the very severe acidemia.

There was no constant pathognomonic clinical presentation by which one could predict the drug invoked. Salicylate usually presented with the patient not in coma, hyperventilating, vomiting and complaining of tinnitus. Features which were confined to specific drugs, but which were not present in every case, are shown in

Table VI. One quarter of the patients who had taken Mandrax (methaqualone plus diphenhydramine) had bizarre signs of CNS irritability which occasionally confused the diagnosis. Although these signs had been reported to occur with large doses of methaqualone alone,[17] these effects may be potentiated by the addition of diphenhydramine which is known to lower seizure threshold.[18] Cardiotoxicity of the tricyclic antidepressants has been reported[19] and is supported by our experience. Problems of significant cardiac conduction blocks and ventricular arrhythmia occurred in 25% of those patients who have taken a tricyclic antidepressant overdose. This was in contrast to our experience with cardiotoxicity in the remainder of the patients in whom we could find only one case (a mono-amine oxidase inhibitor ingestion) in which a significant arrhythmia occurred unexplained by underlying cardiac disease. It is because of this seemingly specific cardiotoxic risk that we routinely admit patients with tricyclic intoxication for cardiac monitoring even though the patient may not be in coma.

TABLE VI

CHARACTERISTIC SIGNS OF TOXICITY

Drug	Total Patients	Patients with Signs	Type of Abnormal Signs
Mandrax (methaqualone & diphenhydramine)	25	6	unequal pupils, clonus, hypertonia, hyperflexia
Tricyclics	27	7	cardiac conduction blocks, ventricular arrhythmia

In the 88 deeply unconscious patients who survived, the time of reported ingestion to the time of treatment was less in the group in coma with response to pain, and may account for the less severe clinical state (Table VII). The duration of unconsciousness averaged approximately 10 hours in both the patients with and without response to pain. Only 27 patients (32%) were comatose for more than 12 hours. All patients who were unconscious for more than 12 hours had taken a non-barbiturate hypnotic, a short acting barbiturate, or more than one formulation (Table VIII). The non-barbiturate hypnotics, mainly Mandrax® and methyprylon, were implicated in a disproportionate 45% of the total. Over half of the patients unconscious for more than 12 hours did not have gastric lavage or activated charcoal or were treated more than 12 hours after the reported intake. It is difficult to compare our results in terms of length of coma with those of others when it is dependent on the type of drug taken as well as other variables. Lawson reports that in 170 comatose patients, 80 (47%) were unconscious more than 12 hours and 25 (20%) for more than 24 hours.[3] We attribute part of the apparent shorter duration of coma and possibly the lower mortality in our patients to the routine use of activated charcoal after gastric lavage.

TABLE VII

DURATION OF UNCONSCIOUSNESS IN 88 PATIENTS ADMITTED IN COMA

	Average Time		Patients Conscious in Time Interval		
	Intake to Treatment (hrs ± S.D.)	Until Conscious (hrs ± S.D.)	0-12 hr.	12-24 hr.	24 hr.
Coma Without Pain Response	13.2 ± 7.1	9.2 ± 8.1	21	7	5
Coma With Pain Response	8.5 ± 6.2	10.1 ± 6.2	42	13	2
Total			63	20	7

TABLE VIII

DRUGS TAKEN BY 27 PATIENTS IN COMA LONGER THAN 12 HOURS

Drug	Number of Patients	
Non-Barbiturate Hypnotics:		
Mandrax	5	45%
Methyprylon	5	
Others	2	
Barbiturates	7	
Multiple Drugs	8	

Although our clinical service is backed by an effective diagnostic toxicological laboratory with measurements available on a 24-hour basis, emergency identification of the causative drug only occasionally led to specific therapy (Table IX). Of the 22 cases in which toxicology appeared to influence treatment, 14 were salicylate intoxication, 2 were bromide, 2 were methanol, 1 was cyanide and 3 were tricyclic antidepressants. The majority of these patients fell into the group which were not in severe coma. Quantitative toxicology was obviously most readily available in the same group. Qualitative toxicology was rarely requested on an emergency basis, rarely alterated therapy and would therefore appear to be of little or no general use. These data, however, are extremely important in the context of this report since the tenacity of our reported epidemiology could be questioned were the history of drug ingestion not confirmed by toxicology in the majority of patients. It is also important that the highest percentage of completed toxicology and the highest percentage in which toxicology verified the history occurred in the group of patients in most severe coma. It is a credit to our toxicology laboratory in view of the rather limited facilities that drug ingestion history was verified in such a high percentage of cases. Our experience is even more favourable when compared to a report of 65-85% positive identification of drug in intoxications using mass spectrometry.[20]

TABLE IX

RESULTS AND INFLUENCE OF TOXICOLOGY COMPLETED IN 128 OF 169 PATIENTS

	Coma Without Pain Response %	Coma With Pain Response %	Semicoma or Conscious %
Toxicology Completed	90	83	61
Toxicology Verified History	91	84	76
2 or More Drugs Verified	36	33	8
Quantitative Toxicology	36	37	49
Toxicology* Influenced Treatment	12	8	35

*Salicylate 14 of 22

DISCUSSION

Many of our patients had a history of previous drug overdose or attempted suicide by other means. Only 32% of these patients were under present psychiatric care. These are disturbing facts and suggest that more attention be paid to prevention of drug overdose through better recognition of the vulnerable patient, especially more intensive continuing care of repeaters.

The results of treatment of drug overdose in our patients support the well-established principle of supportive care as the basis of successful therapy. This care must often include nursing of the unconscious patient, supportive adequate respiratory function and assessment and correction of acid base and electrolyte disturbances. The routine use of large amounts of activated charcoal after gastric lavage may play a critical role in the encouraging prognosis as reported herein. In terms of the influence of toxicologic analysis on care of the patient, it would appear that relatively simple assays of a very limited number of drugs is all that is required outside the areas of research.

Patients intentionally taking an overdose of drugs appear to take any drug that is readily available to them. Any of a variety of drugs can cause death if the patient does not obtain prompt medical care. However, the hypnotic drugs, barbiturate and non-barbiturate, cause the most serious states of overdose if measured by the length of unconsciousness after starting treatment. The prolonged coma in such patients is probably explained by the narrow therapeutic range which is characteristic of these drugs, *i.e.* a small difference between the dose which induces sleep and that which causes coma. Careful comparative studies should be made of the efficacy of other drugs as nighttime sedatives including the benzodiazepines. If such drugs prove as effective as the hypnotic group, less risk as far as severe overdose is concerned would attend their use.

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Factors Affecting the Pharmacokinetics of Some Psychoactive Drugs

William J. Jusko, Ph.D.

ABSTRACT An overview of the pharmacokinetics of psychoactive drugs reveals that, with the exception of compounds classified as psychomotor stimulants and anorectic agents, psychotherapeutic drugs have many common physicochemical and pharmacokinetic properties. They tend to be appreciably lipid-soluble compounds which accounts for their rapid uptake into the central nervous system, extensive degree of biotransformation, and lack of significant excretion into urine. These drugs are often bound appreciably to plasma proteins. Patients commonly exhibit a long half-life and large volume of distribution of these agents and, related to the preceding factors, usually show a large degree of intrasubject variation in their distribution, metabolic clearance, and steady-state plasma drug levels.

A physiological pharmacokinetic model was used to simulate the temporal behavior of a hypothetical drug with the general characteristics of a psychoactive agent. This model shows the role of factors such as rate of hepatic metabolism, liver blood flow, protein binding, body fat mass, and perfusion rates of various tissues in explaining some of the pharmacokinetic characteristics of psychotherapeutic drugs.

Psychotherapeutic drugs represent the most widely used, abused, and misused class of pharmacological agents. The proper employment of antidepressant drugs has been said[1] to have "revolutionized the practice of psychiatry" while, at the same time, the immense street traffic in psychoactive agents has evolved many well-known medical and social problems.

Chronically-administered psychoactive drugs are often improperly used, not only because of hasty consideration of their indications and risk-benefit ratio[1,2] but because the variability in pharmacokinetic properties of most of these drugs makes the use of standard dosage regimens a matter of chance as to whether a given patient will be maintained at a body level which provides a therapeutic and nontoxic effect from the drug. The purpose of this report is to provide an overview of the

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pharmacokinetics of psychotherapeutic agents with respect to considering the typical distribution and disposition characteristics of such drugs and the factors which cause inter-subject variability in the relationship between the maintenance dose and the steady-state plasma concentration of a drug.

PHARMACOKINETIC PROPERTIES

Mathematical Relationships

In order to discuss the pharmacokinetic properties of any drug, it is essential that some of the basic mathematical parameters and relationships be defined and described. The biological half-life ($t½$) of a drug is the most frequently used pharmacokinetic parameter. After single intravenous doses, most psychoactive drugs show multi-exponential curves for disappearance from plasma. Examples from the literature are available for drugs such as diazepam,[3] pentobarbital,[4] tetrahydrocannabinol,[5] morphine,[6] and reserpine.[7] The early phase of disposition reflects the occurrence of both distribution into tissues and true elimination from the body. The later phase, often called the beta-phase, provides a useful biological half-life because, as will be shown, it is this half-life which determines the disposition rate of the drug from the body after attainment of the steady-state.[8]

The apparent volume of distribution (V_D) is an often misunderstood and miscalculated parameter. This report will only be concerned with the steady-state volume of distribution, *i.e.;* the parameter which is the proportionality factor between the amount of drug in the body (X_B^{ss}) and the mean plasma concentration (C_p^{ss}) at the steady-state:

$$X_B^{ss} = V_D \cdot C_p^{ss} \qquad \text{(Eq. 1)}$$

For drugs which are eliminated very slowly, the situation is simplified in that several of the alternative V_D parameters are mathematically nearly identical to the steady-state value.[9] Thus reasonable estimates of V_D *for a drug with a long half-life* are:

$$V_D \cong \frac{D_o}{B} \cong \frac{D_o/t½}{\text{Area}\ (0.693)} \qquad \text{(Eq. 2)}$$

where D_o is the dose, B is the plasma drug concentration when the terminal disposition phase is extrapolated to time zero, and Area is the total area under the plasma concentration versus time curve.

The metabolic or plasma clearance (Cl_M) of the drug is an extremely useful parameter because it not only reflects the removal rate of a drug, but provides the proportionality factor between the dose (D_o), bioavailability (F), dosing interval (τ), and the mean plasma concentration at the steady-state[10,11]

$$C_p^{ss} = \frac{F.D_o}{Cl_M \cdot \tau} \qquad \text{(Eq. 3)}$$

Thus, knowing the bioavailability and plasma clearance of a drug, one can devise a dosage regimen (D_o,τ) which will generate a pre-selected plasma drug concentration for a patient. The value of Cl_M can be calculated from:

$$Cl_M = 0.693.V_D/t½ \qquad \text{(Eq. 4)}$$

The bioavailability of a drug represents the fraction of the dose which reaches the plasma. It is calculated from the plasma concentration areas after administration of oral (po) and intravenous (iv) doses of a drug:

$$F = \text{Area}_{po}/\text{Area}_{iv} \qquad \text{(Eq. 5)}$$

Some of the psychotherapeutic drugs are subject to the first-pass effect after oral dosing. This term reflects the fact that these drugs have a high hepatic extraction ratio and an appreciable fraction of an oral dose of the drug is metabolized as the portal blood passes through the liver before mixing with the general circulation. In such cases, the following equation often yields an accurate bioavailability value:[12]

$$F = 1 - \frac{\text{Dose}_{iv}}{Q_L \cdot \text{AUC}_{iv}} \qquad \text{(Eq. 6)}$$

where Q_L is the plasma or blood flow through the liver.

Properties of Some Psychoactive Drugs

A summary of the physicochemical and pharmacokinetic properties of a number of psychotherapeutic drugs is provided in Table I. Calculations of the various parameters in Table I were made either in the original reports or were estimated by the author using the previously described pharmacokinetic relationships. The agents listed are typical of the spectrum of psychotherapeutic drug classes, including tranquillizers (diazepam and meprobamate), sedatives (pentobarbital), hypnotics (glutethimide), antipsychotics (chlorpromazine), antidepressants (nortriptyline and imipramine), narcotic analgesics (morphine, methadone, and tetrahydrocannabinol), and an adrenergic depleting agent (reserpine). A major class of agents which is excepted from this review are the psychomotor stimulants and anorectic agents. A brief contrast of their properties will be made below.

The drugs listed in the Table show several physicochemical properties in common. Their partition coefficients (octanol:water) are usually appreciable, indicating that lipid uptake of the drugs should be substantial. Indeed, direct observation of drug uptake by adipose tissue has been made in some instances, including for diazepam[38] and tetrahydrocannabinol.[32] The pKa of many of the compounds is within 1 or 2 units of pH 7.4 and thus the degree of ionization at physiological pH may often affect the transfer of many of these drugs across certain membranes. For example, the urinary excretion of imipramine is pH-dependent, although the fractional elimination of the drug by this route is extremely small and this phenomenon is of little clinical importance.[39] The binding of the drugs to plasma proteins is often appreciable but is also usually somewhat variable. For example, the binding of chlorpromazine varies from 91 to 99 percent[24] while desmethylimipramine binding varies from 87 to 95 percent in treated patients (Figure 1).

The psychotherapeutic agents listed in Table I show pharmacokinetic similarities in their half-lives, in that they tend to be fairly long, and in the elimination process, which usually involves nearly complete biotransformation by oxidative pathways. The extensive metabolism, as well as the appreciable protein binding of the respective drugs, are consistent with the lipid-solubility of the compounds, since these characteristics of drugs usually correlate in structure-activity studies.[41] Also, the high lipid solubilities of drugs results in their reabsorption from the urine and

TABLE I

PHYSICOCHEMICAL AND PHARMACOKINETIC PROPERTIES OF SOME PSYCHOACTIVE DRUGS

Drugs	APC*	pKa	FB†	Percent Bioavailable	t½, hr.‡	V_D liters§	Cl_M, ml/min. ¶	Percent Metabolized	Metabolic Pathways	References
Diazepam	180	3.4	.93	100	9-42 27-36	11-83 161-205	12-41	100	Demethylation Hydroxylation	3, 13-16
Nortriptyline	—	—	.945	56-70	19-93	20-56	290-840	~100	Hydroxylation Demethylation	17-20
Imipramine	200	9.5 8.0	.958	—	24	—	—	~100	Demethylation Hydroxylation	21-23
Chlorpromazine	841	9.3	.91-.99	30	30	780	300	70	Hydroxylation Demethylation	21, 24-26
Meprobamate	80	—	.40	—	11-15	54	43	—	Hydroxylation	13, 27
Pentobarbital	19	8.03	.70	98	42, 46	134	26	> 90	Hydroxylation	4, 28-29
Glutethimide	—	11.2	.54	—	40 5-22	—	—	—	Hydroxylation	30-31
Tetrahydrocannabinol	very high	—	high	—	56	660	136	100	Hydroxylation	5, 32-33
Morphine	—	—	high	‖	10-44	700	500	~100	Demethylation Glucuronidation	6, 34
Methadone	—	—	.87	70	10-47	93-300	17-83	50-70	Dealkylation	35-37
Reserpine	—	—	—	‖	271	—	—	~100	Hydrolysis Demethylation	7

* APC = Apparent partition coefficient between octanol and pH 7.2 or pH 7.4 buffer, except for pentobarbital where chloroform value is listed.

† Fraction bound to human plasma proteins, except for diazepam and meprobamate (dog plasma) and pentobarbital (4% bovine albumin).

‡ t½ = Biologic half-life, representing the value for the terminal portion of the plasma concentration versus time curve.

§ V_D= Volume of distribution, either in the steady-state or during the slowest phase of elimination.

¶ Cl_M = Plasma clearance. ‖ Bioavailability is probably incomplete.

bile, another factor predisposing them to metabolism as a major route of elimination.

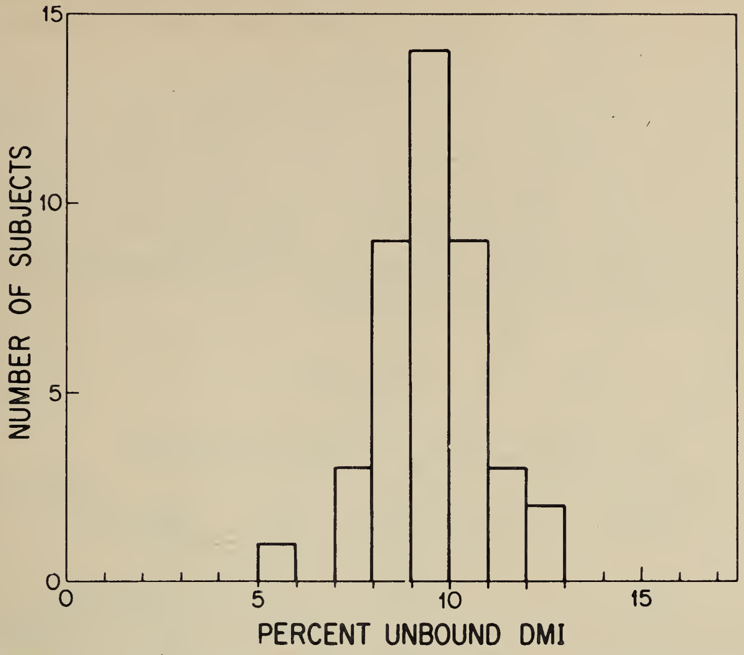


Figure 1. *Variability in plasma protein binding of desmethylimipramine in a group of patients. The free drug concentration varies from 5 to 13 percent of the total plasma concentration. Data from Sjoqvist et al.*[40]

The half-lives, volumes of distribution, and plasma clearances of the drugs in Table I are variable, both among the compounds and for any individual drug in a group of patients. The metabolic clearances of the drugs, for example, range from a low of about 26 ml/min for pentobarbital, to fairly high values of over 800 ml/min for nortriptyline. The latter represents nearly complete hepatic extraction of all drug in the blood perfusing the liver and is nearly a maximum hepatic clearance value for any type of drug.

TABLE II

VARIABILITY IN DIAZEPAM PHARMACOKINETICS*

Patient	Cp^{ss} mg/ml	t½, hr.	V_D, l.	Cl_M, ml/min.
K	1.4	9	11	15
DS	0.5	23	83	42
dB	1.5	24	29	14
vT	0.7	30	78	30
V	1.3	42	59	16

* From van der Kleijn;[14] Dose = 10 mg. tid.

The variability in disposition of diazepam among a group of patients was studied by van der Kleijn[14] and is shown in Table II. At a dose of 30 mg/day, it can be seen that these adult subjects show a three-fold range of steady-state plasma concentrations and clearance values. However, the t½ and V_D values are considerably more erratic, having a 5- to 8-fold range.

One source of variable plasma levels and clinical response to drugs with high clearance values is the first-pass effect. For example, the bioavailability of nortriptyline has been studied by Alexanderson *et al.*[20] as shown in Table III. Only about 60 per cent of an oral dose reaches the plasma and the use of Equation 6 provides a good estimate of bioavailability. Other drugs which would be expected to be subject to an appreciable first-pass effect are chlorpromazine and morphine because of their high clearance values (Table I).

TABLE III

BIOAVAILABILITY OF NORTRIPTYLINE*

Subject	Route of Admin.	Area hr. ng./ml.	Bioavailability Observed	Predicted†
1	im	1310		70
	po	854	65	
2	im	636		38
	po	445	70	
3	im	1020		60
	po	571	56	

* From Alexanderson *et al.*[20]
† Using Equation 6.

The psychomotor stimulants and anorectic agents such as the amphetamines differ from most of the other psychoactive drugs in that they have shorter half-lives and a greater part of the dose is excreted by the kidneys. For example, the half-life of dexamphetamine ranges from 4 to 13 hours and the percentage of the dose excreted in urine, which is subject to pH-dependent tubular reabsorption, ranges from about 3% at alkaline pH to 54% at acid pH.[42,43] Van Rossum has listed the biological half-lives for some other drugs in this class.[44]

Nonlinear Pharmacokinetics

There are a few drugs which show saturability in their rate of metabolism as body levels are increased. Ethanol,[45] salicylate,[46] and diphenylhydantoin[47] are examples of such compounds. Plasma level versus dose data from a group of patients treated with the anticonvulsant agent, diphenylhydantoin, are shown in Figure 2. Most drugs exhibit a linear relationship between the dose and the steady-state blood level.

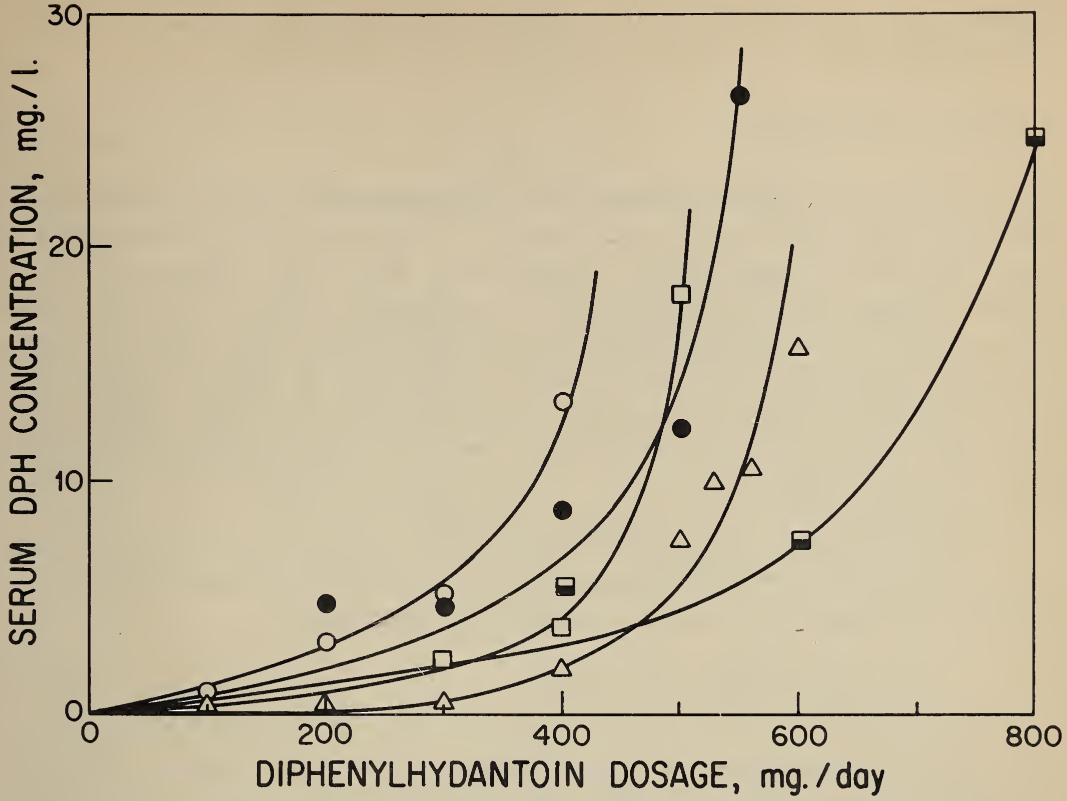


Figure 2. *Nonlinear relationship between daily dosage and steady-state plasma concentrations of diphenylhydantoin in epilepsy patients. Data from Bochner et al.*[47]

Compounds such as diphenylhydantoin pose therapeutic problems because of the steep rise in plasma drug levels at dosage increments in the therapeutic range. This factor, plus the large variability in pharmacokinetics among subjects, accounts for the necessity of monitoring serum concentrations of diphenylhydantoin in order to maintain patients in the usual therapeutic range of about 10 to 20 mg/liter.[48]

The opposite phenomenon seems to occur with the phenothiazine derivative, thioridazine. This compound shows proportionately lower plasma levels at larger doses in man.[49, 50] The reason for this effect is not known.

PHYSIOLOGICAL PHARMACOKINETIC ANALYSIS

Physiological pharmacokinetics refers to the quantitative utilization of the major physiological functions and body regions affecting drug uptake in mathematically describing the time-course of drug disposition. This approach provides a more realistic basis for considering the body's handling of drugs than the methods of classical compartmental pharmacokinetics. The practical use of physiological pharmacokinetics was essentially pioneered by Bischoff and Dedrick in describing thiopental distribution and elimination[51] and has since been successfully applied in studies with methotrexate[52] and arabinosylcytosine,[53] among other drugs. The present use of similar methodology is to simulate the expected pharmacokinetic effects of variability in common patient-drug factors such as hepatic biotransformation capability, liver blood flow, protein binding, and adipose mass using a drug model reflecting the properties of many of the psychotherapeutic agents.

Description of the Model

The physiological model which was used is represented in Figure 3. This scheme shows the major body regions known to be of importance in the distribution of most drugs. Each tissue region is divided into three water compartments: the capillary plasma volume where the drug is in equilibrium with the rest of the tissue, the interstitial water, and the cells and cell water. Each tissue region receives a designated plasma flow and red cell uptake of the drug is ignored. Because most psychotherapeutic drugs have negligible renal excretion, the kidney region of the model was not used except that its plasma volume was added to the central plasma pool. The numerical parameters were essentially adapted from Bischoff and Dedrick for a 70 kg. man. [51-53]

The mathematics of this type of system were adapted from the description of Bischoff and Dedrick.[52] A mass balance equation was set up for each tissue in the manner, for example, for muscle concentration of drug (C_M):

$$V_M \cdot \frac{dC_M}{dt} = Q_M \cdot (C_p - C_{me}) \qquad \text{(Eq. 7)}$$

where V_M is the muscle volume, Q_M is the plasma flow to the muscle, and C_p and C_{me} are the tissue inflow and outflow plasma concentrations of drug, respectively. The value of C_{me} was calculated based on the tissue model shown in detail in Figure 4. The free drug in cell water was assumed to equilibrate with the free drug in interstitial fluid and plasma and drug in the latter two fluids was assumed to be partly bound to proteins. For simplicity, linear binding of the drug was assumed as described by:[54]

$$S = n.k.P_t = \frac{F_b}{1-F_b} \qquad \text{(Eq. 8)}$$

where S (the product of n, the number of binding sites; k, the drug-protein association constant; and P_t, the protein concentration) can be estimated from F_b, the fractional binding of the drug. Values of F_b were assumed for plasma, and interstitial binding was then estimated from the protein concentration in each tissue. The liver interstitial protein concentration was set identical to that of plasma, the central nervous system value was set as zero, and all other tissues were given values one-half of the plasma protein concentration. These interstitial protein concentrations are based on measurements in lymph draining each type of tissue.[55] Values of S could be adjusted proportionately to estimate the fractional binding for each tissue. Thus the fractional binding of the drug in muscle interstital fluid (F_{im}) was obtained from:

$$F_{im} = \frac{0.5.S}{1 + 0.5.S} \qquad \text{(Eq. 9)}$$

The value of C_{me} was obtained by initially calculating the total amount of drug in the tissue (A_M), viz:

$$A_M = C_p \cdot V_{pm} + C_M \cdot V_M \qquad \text{(Eq. 10)}$$

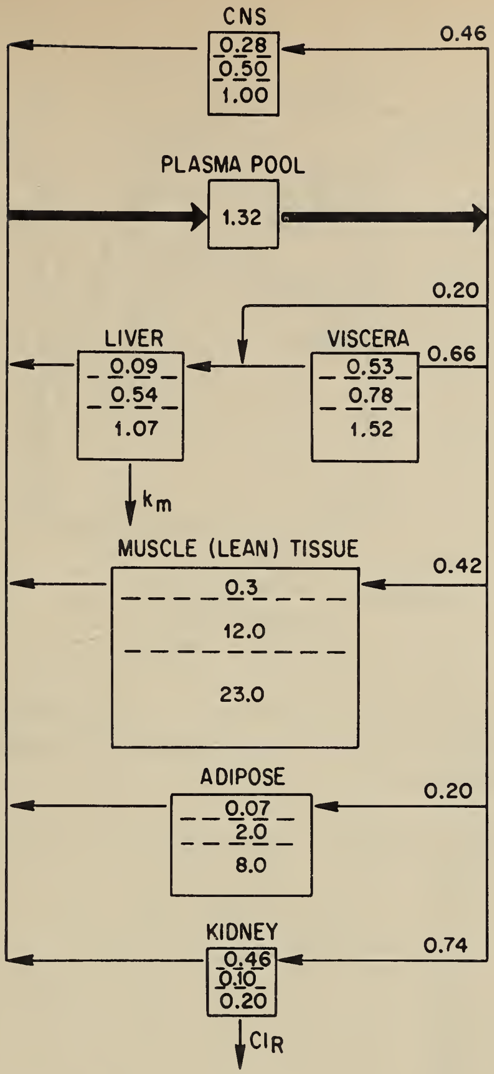


Figure 3. *Major body regions accounting for blood flow and drug distribution and elimination. The numbers on the arrows are the plasma flow rates and the numbers in the tissue compartments are the capillary plasma volume in equilibrium with the tissue (upper value), interstitial fluid volume (middle value), and cell water or adipose volume (lower value).*

where V_{pm} is the plasma volume equilibrated with the tissue. Then redistribution of drug between the three compartments was calculated from the volume of the tissue and the binding of the drug. For example, the drug concentration in plasma leaving the muscle is:

$$C_{me} = \frac{C_w}{1-F_b} = \frac{A_T}{(1-F_b)\left[\frac{V_{pm}}{1-F_b} + \frac{1-0.66\,F_{im}}{1-F_{im}} \cdot V_M\right]} \qquad \text{(Eq. 11)}$$

where C_w is the drug concentration in plasma water.

All computations were carried out by numerical integration of the series of equations such as Eq. 7-11 using the MIMED computer program.[56] In addition to

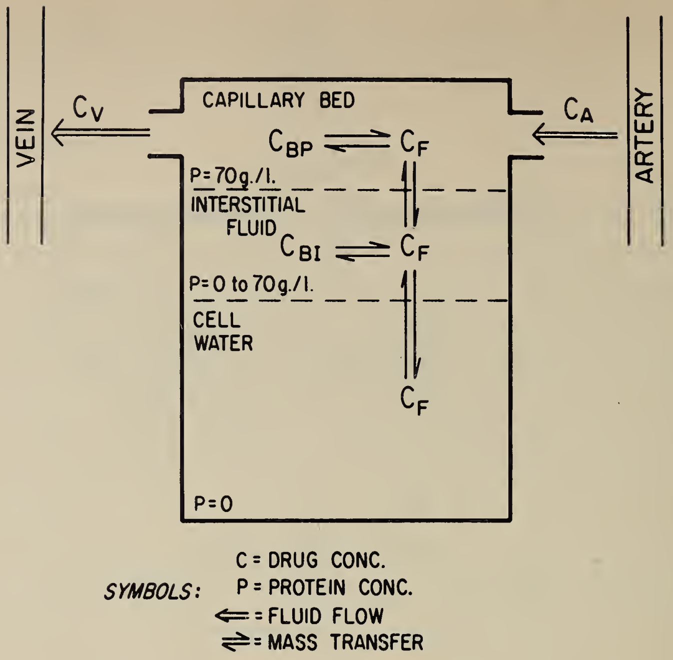


Figure 4. *Detailed model of an individual tissue showing equilibration of the unbound drug (C_F) between water compartments and proteins in the plasma (C_{BP}) and interstitial fluid (C_{BI}).*

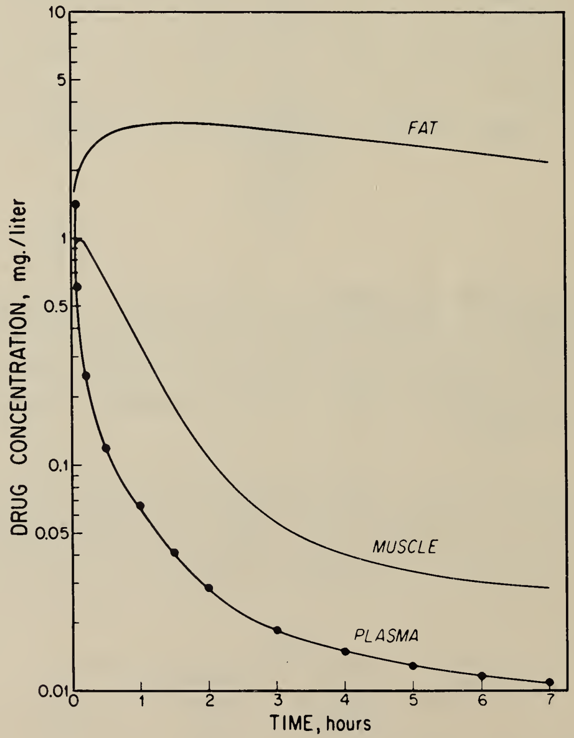


Figure 5. *Time course of calculated drug concentrations in plasma, muscle, and fat showing the role of blood flow, tissue size, and tissue binding (lipid partitioning) in determining the time course of drug uptake and loss.*

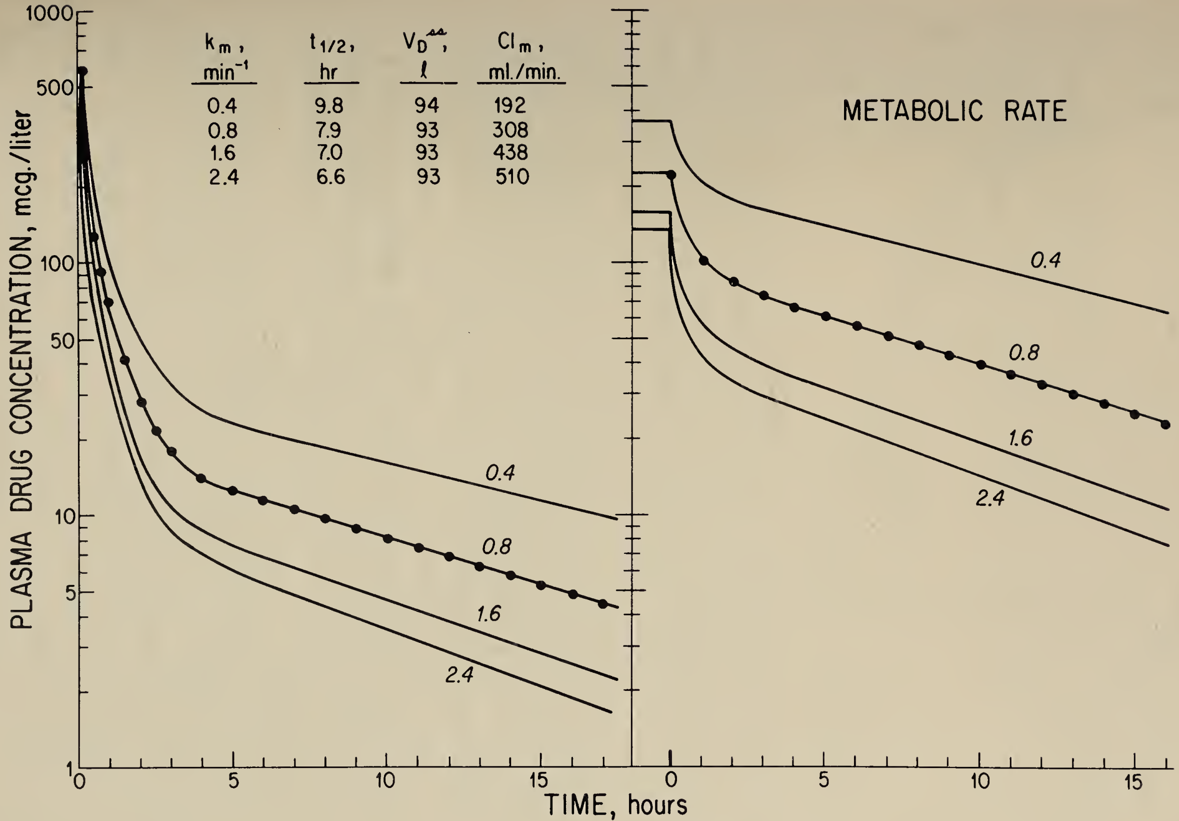


Figure 6. *Effect of intrinsic metabolic rate on the time-course of plasma drug concentrations after a single 10 mg. dose (left-hand side, LHS) and following attainment of a steady-state with a dosage regimen of 100 mg./day (right-hand side, RHS). This is a digital computer simulation based on the model shown in Figures 3 and 4. The value of* k_m *was varied as designated and the listed parameters were calculated from the data using Equations 1-4 in the text.*

using the physiological parameters shown in Figure 3, the computations involved use of a hypothetical drug which had the standard characteristics of being entirely metabolized by the liver with a *hepatic* rate constant of 0.8 min^{-1}, 90 per cent bound to plasma proteins, and lipid soluble with an adipose: plasma water partition coefficient of 100. This description is generally similar to many of the psychoactive drugs listed in Table I and the data simulations should thus show the type of behavior which might be expected when one of the basic pharmacokinetic or physiological parameters is altered or is different among patients.

The data simulations were performed under two dosing situations. The time-course of drug levels in various tissues was calculated: (1) after a 10 mg. intravenous dose was injected over a 1 minute period and, (2) after the system was allowed to reach a steady-state with a dosing input of 100 mg./day which was then discontinued.

Differences in Tissue Distribution

Figure 5 shows the expected time-course of drug levels in plasma, muscle, and adipose tissue after a 10 mg. intravenous dose of drug. The curvilinear decline in plasma drug concentrations is typical of all psychoactive drugs after administration of a single dose. Muscle, which is a large mass of relatively poorly perfused tissue, shows a delay in attaining a peak concentration after the dose and subsequently shows a decline in drug concentrations which parallels the plasma curve. Adipose tissue, which is one of the most poorly perfused tissues, reaches a peak drug concentration at a much later time but also maintains a high drug concentration because of the lipid solubility of the drug. These tissues, because of their large size and low perfusion rate, determine the terminal rate of drug disposition from the body by their rate of release of the major fraction of the residual drug in the body. The central nervous system (CNS) is highly perfused (Figure 3) and readily penetrated by lipid-soluble drugs. The drug concentration in the CNS is thus attained and dissipated fairly rapidly. This accounts for the quick onset of effect of intravenously-administered psychoactive drugs.

Role of metabolic rate. – The time-course of single dose and post-steady-state plasma concentrations of drug are shown in Figure 6 at several designated k_m values. As expected, the larger k_m value results in more rapid disappearance of the drug from the system. The plasma clearances (CI_M) increase nearly in proportion to the k_m values, although the half-lives (t½) are changed only slightly. The steady-state volume of distribution (V_D^{ss}) is not affected. The steady-state plasma concentrations change in inverse proportion to CI_M as anticipated from Equation 3. As pointed out previously by Gibaldi and Weintraub,[8] the terminal (beta) half-life after the single dose will reflect the post-steady-state half-life of the drug. Thus it is extremely important to follow plasma levels of psychoactive drugs for a sufficiently long time to allow characterization of the full disposition curve.

Role of hepatic blood flow. – The time-course of single dose and post-steady-state plasma concentrations of drug are shown in Figure 7 at several hepatic flow rates, both greater than and less than normal. These large alterations in blood flow produce definite changes in the half-life, metabolic clearance, and steady-state plasma levels, but no change occurs in the volume of distribution in this test system.

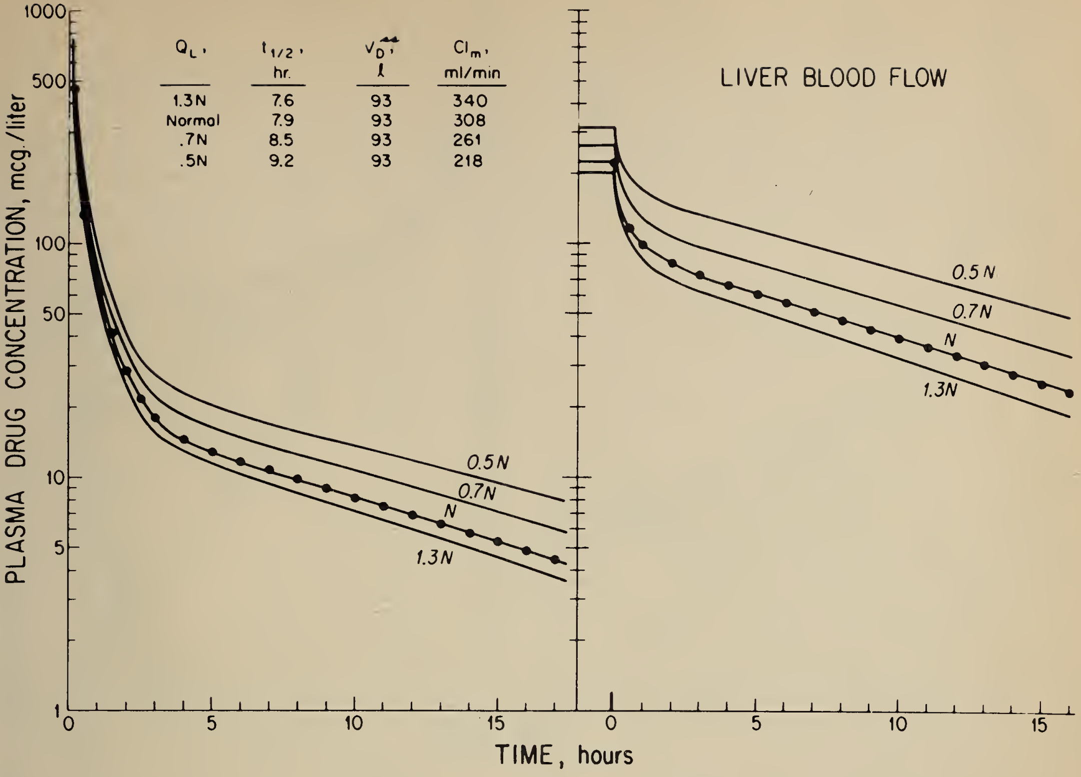


Figure 7. *Effect of hepatic plasma flow on the time-course of plasma drug concentrations after a single dose (LHS) and after attainment of steady-state (RHS). The data were generated as described in Figure 6 except that* k_m *was maintained constant at 0.8 min.*$^{-1}$ *while plasma flow was varied as designated.*

Role of protein binding. – The time-course of single dose and post-steady-state plasma concentrations of drug are shown in Figure 8 as the fractional plasma (and tissue) binding of the drug is modified. Such differences in fractional binding could be caused by changes in protein concentrations, drug-protein association constants, or presence of binding inhibitors. Tissue binding was also assumed to be affected as described by Equation 8. Increased binding has a biphasic effect on disappearance of the drug from plasma as seen from the curves. During the early time-period, the greater fractional binding causes slower disposition from plasma; later, the disposition is relatively more rapid. The latter phenomenon is of greater importance in the multiple-dosing situation. An additional complexity is the fact that V_D^{ss} also decreases as F_b increases. Thus the greater degree of binding causes more drug to be maintained in the plasma compartment which, in turn, increases the disappearance rate. An unexpected effect is the relatively small change in CI_M values, but this is consistent with the direct relationship between V_D and t½ values shown in Equation 4. The steady-state drug levels are affected very little by the change in binding.

The same type of phenomenon has been proposed to account for differences in pharmacokinetics of chlorpromazine among species[26] and of propranolol among subjects.[57] Since the present model was developed by assuming that the metabolic rate constant was related to the total drug concentration in the liver, perhaps a different type of behavior pattern will be evident if it is assumed that hepatic

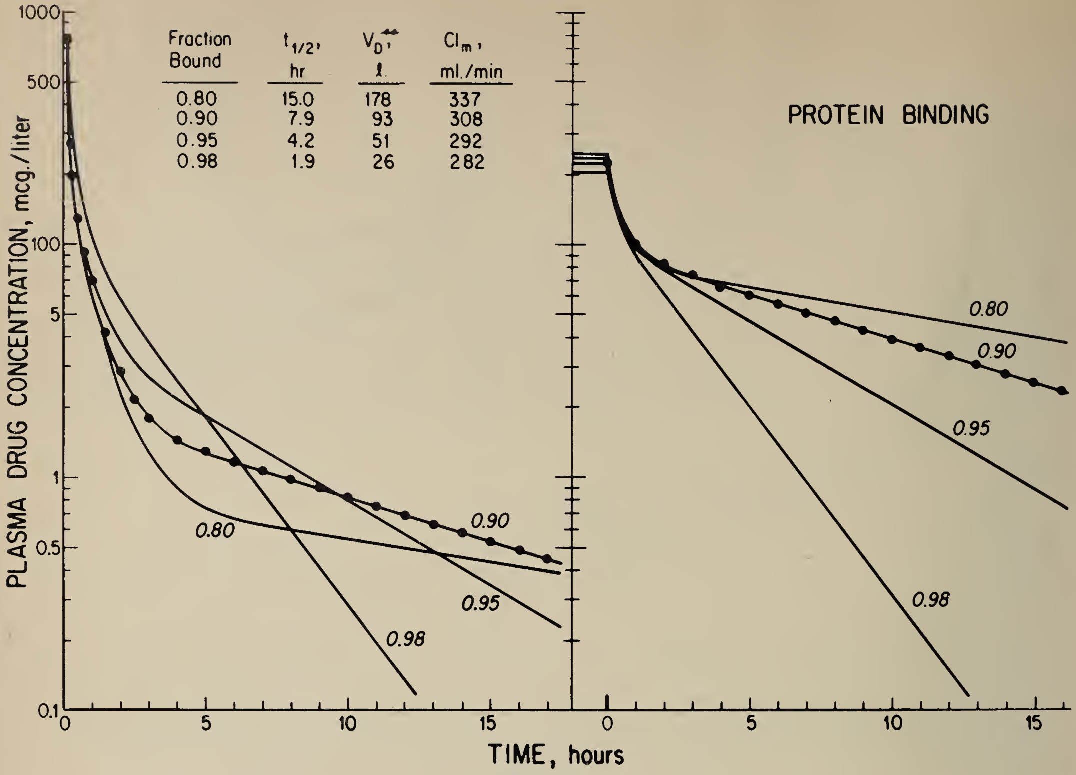


Figure 8. *Effect of plasma protein binding on the time-course of plasma drug concentrations after a single dose (LHS) and after attainment of steady-state (RHS). The data were generated as described in Figure 6 except that* k_m *was maintained constant at 0.8 min.*$^{-1}$ *while plasma and interstitial protein binding of the drug was changed as designated.*

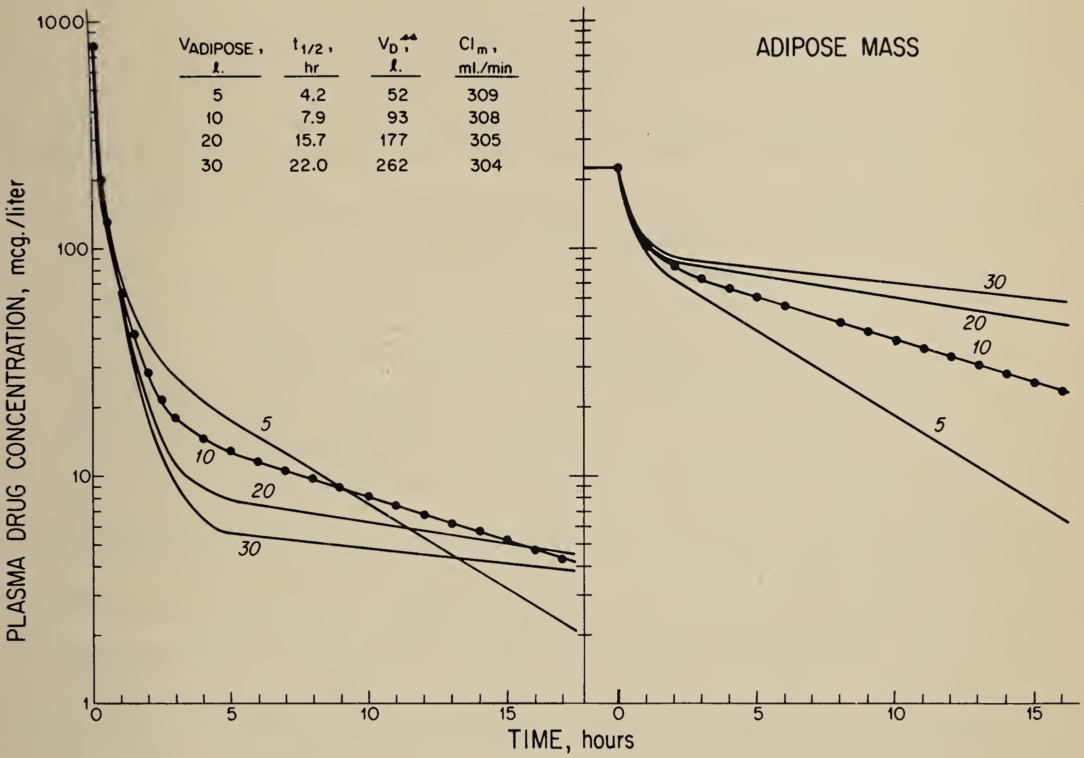
metabolism is governed by the free drug concentration in the liver. This aspect will be tested in the future.

Role of adipose mass. – The time-course of single dose and post-steady-state plasma concentrations of drug are shown in Figure 9 as the adipose mass of the hypothetical subject was used as the variable parameter. Because the drug was assumed to be highly lipid soluble, adipose mass becomes an important factor governing the half-life and volume of distribution of the drug. The changes in CI_M and C_p^{ss} were negligible. This type of observation is important in treatment of drug intoxication of obese subjects because they will retain the drug much longer than thin patients and thus require prolonged management.

OVERVIEW AND CLINICAL IMPLICATIONS

Use of a physiological pharmacokinetic model has allowed examination of the effect that individual physiologic and pharmacologic factors have on the overall pharmacokinetics of a psychoactive-like drug. Changes in metabolic rate, hepatic blood flow, protein binding, and adipose mass induce alterations in one or more pharmacokinetic parameters which govern the dose versus steady-state plasma level relationship of drugs. These modifications in plasma concentrations of the drug indicate that physiologic models, to accurately characterize drug disposition data, require fairly realistic estimates of the major patient-drug characteristics.

Pharmacokinetic studies are often performed in twins and non-twins to discern genetic influences on drug disposition.[19] The present data indicate that nearly identical plasma level curves in twins is not only the result of genetic similarities in metabolic capability, but similarity in body size, proteins, and adipose mass are of equal importance.



$V_{ADIPOSE}$, ℓ.	$t_{1/2}$, hr	V_D, ℓ.	Cl_m, ml./min
5	4.2	52	309
10	7.9	93	308
20	15.7	177	305
30	22.0	262	304

Figure 9. *Effect of adipose mass on the time-course of plasma drug concentrations after a single dose (LHS) and after attainment of steady-state (RHS). The data were generated as described in Figure 6 except that k_m was maintained constant at 0.8 min.$^{-1}$ while the adipose mass was changed as designated.*

The pharmacokinetics of most psychoactive drugs show appreciable variability among patients with some of the reasons made evident in this report. The most important pharmacokinetic measurements which should be made during the course of single-dose studies are the metabolic clearance and the bioavailability. Knowledge of the relative value and variability of these parameters will allow accurate estimation of the steady-state plasma concentrations of drug which will be achieved during multiple-dosing. The clinical usefulness of psychotherapeutic drugs can also be improved by further studies relating the steady-state plasma concentration to the therapeutic and toxicological effect of the drug.[58] Thus optimum drug efficacy and safety will be achieved through utilization of clinical pharmacokinetics for devising dosage regimens based on pharmacokinetic principles and using serum concentration measurements for feedback control of therapy.

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Factors Influencing the Interpretation of Drug Concentration in Acute Drug Overdose

Edward M. Sellers, M.D., Ph.D., F.R.C.P.(C)

ABSTRACT The therapeutic effectiveness of antiarrhythmics, antiepileptics, antidepressants and cardiac glycosides has been substantially improved by studies which have defined their therapeutically effective or toxic range of serum concentration. For most psychoactive drugs however, the indications for measuring and use of such concentration determinations is poorly established. Much of the serum concentration data available is based on uncontrolled clinical studies which have failed to recognize the importance of the interval between drug ingestion and the time of sampling and differences between patients.

Epidemiologic studies of the patterns of acute drug ingestion in Toronto have indicated that minor tranquilizers, barbiturates and ethanol account for more than 50% of acute drug ingestions. In addition, approximately 40% of patients who have overdosed have taken more than one drug. The majority of acute drug ingestions are mild and require no treatment. Determinations of serum drug concentrations is not necessary for medical reasons in fully conscious and asymptomatic patients nor will they contribute to patient management when a patient responds to specific therapy, for instance, uncomplicated heroin, morphine or other opiate induced respiratory depressions which is effectively and dramatically reversed by naloxone. There is still need to further reduce the morbidity and mortality due to acute drug ingestion; to develop clinically useful measures of psychoactive drug effect; to define more exactly the relation of concentration and response in serious overdose; to quantitate the importance of individual variations of elimination rates of psychoactive drugs; to determine the frequency and importance of multiple drug ingestion and drug interactions and to study alternative forms of therapy.

Over the past decade the therapeutic effectiveness of antiarrhythmics, antiepileptics, antidepressants and cardiac glycosides has been substantially improved by studies which have defined their therapeutically effective or toxic range of serum concentrations.[1] In contrast to the large individual variation in the optimal dosages of these drugs, the width of their therapeutic serum concentration is relatively narrow. In patients who do not respond to usual drug dosages or who develop unexpected toxicity, in complex clinical situations and in regulation of prophylactic therapy,

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measurement of antiarrhythmic and antiepileptic drug concentrations is essential as a means of adjusting drug dose. For most psychoactive drugs the indications for and use of such concentration measurements is poorly established. Table I summarizes the approximate minimal toxic concentrations of various psychoactive drugs commonly encountered as acute drug ingestions. Much of the serum concentration data available is based on uncontrolled clinical studies which have failed to recognize the importance of the interval between drug ingestion and the time of sampling and differences between patients.

TABLE I

TOXIC CONCENTRATIONS OF PSYCHOACTIVE DRUGS[1, 2]

Drug	Toxic Concentration (approximate lower limit)
Amitriptyline	160 µg/liter
Barbiturates	
Amobarbital	15 mg/liter
Pentobarbital	10 mg/liter
Phenobarbital	60 mg/liter
Secobarbital	10 mg/liter
Bromide	15 mEq/liter
Chlordiazepoxide	10 mg/liter
Chlorpromazine	1 mg/liter
Diazepam	1 mg/liter
Diphenylhydantoin	20 mg/liter
Ethanol	800 mg/liter
Ethchlorvynol	20 mg/liter
Glutethimide	10 mg/liter
Isopropanol	1000 mg/liter
Meprobamate	50 mg/liter
Methanol	1000 mg/liter
Methaqualone	8 mg/liter
Propoxyphene	1 mg/liter
Salicylate	300 mg/liter

RELATION OF DRUG DOSE, SERUM AND PHARMACOLOGIC EFFECT

The enormous individual differences in the relationship between the dosage of a drug, serum concentration and the intensity of its pharmacologic action is due to many factors which influence the relationship shown in Figure 1. The serum concentration resulting from ingestion of a drug is influenced by the bioavailability of the dosage form ingested, by host factors which affect the rate or completeness of gastrointestinal absorption such as gastrointestinal motility, by body size and composition, by distribution through fluid compartments, by binding at inactive sites in serum and tissues and by rates of biotransformation and excretion. All these variables are subject to individual and temporal variations due to consequences of disease and to concomitant administration of other drugs. There are large genetically controlled differences in the rates of biotransformation of drugs, e.g., imipramine,[3] secobarbital.[4] Disease induced disturbances in cardiac, hepatic, and renal function

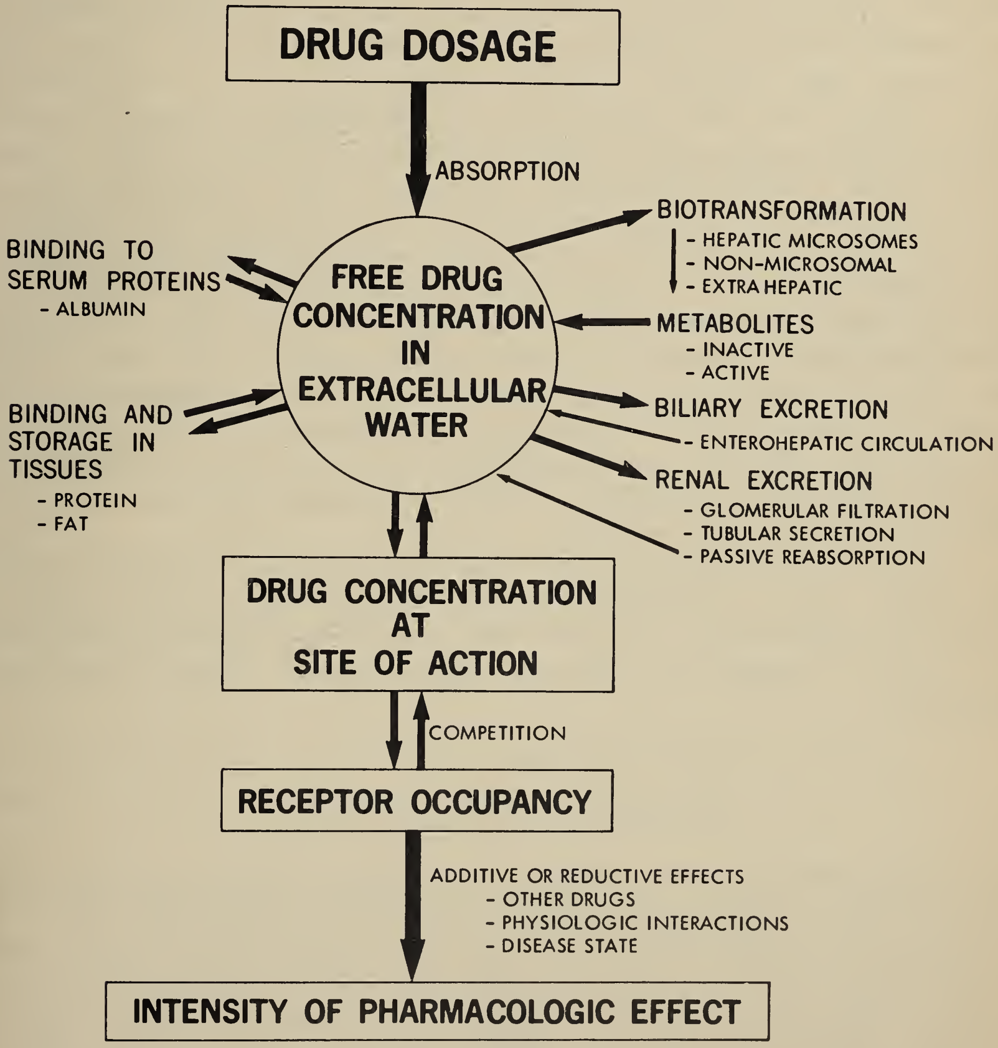

Figure 1. *Relation of Drug Dose, Serum Concentration and Pharmacologic Effect.*

are also responsible for much variation in the biologic half-life of drugs among patients and, with time, in the same patient. Finally, the half-life of some drugs, such as aspirin, ethanol and diphenylhydantoin increases with their dose and serum concentration. Attempts have been made to correlate minimal lethal dose and clinical response in acute drug overdose. Quite understandably these correlations have not been very consistent. Unlike antiepileptics where clinical effect can be measured by determining the number of seizures, or with antiarrhythmics where the abolition of an arrhythmia is an easily measurable end point, with most psychoactive drugs there is no unique end point of effect to measure. Some measures such as electroencephalogram, electrocardiogram, the minute ventilation, tremor, and cardiac output have potential but these indices are not specific and some are not available for routine clinical use.

The influence of acute or chronic concurrent disease in the acute drug ingestion will importantly alter the expected relation of serum concentrations and clinical response. Infection, aspiration, hypovolemia,, hypoxia, hypotension, electrolyte abnormalities, acidosis and alkalosis occur frequently and will alter volumes of drug distribution, organ perfusion and tissue responsiveness. It is to be expected that slight changes in pH will markedly alter the distribution of drugs. For instance salicylate predominantly ionized at pH 7.4, would in acidosis enter cells to a greater extent and be associated with greater toxicity at the same total serum concentration.[5] The influence of chronic liver and renal disease in acute drug ingestion is important for drugs which rely on hepatic biotransformation or renal excretion for their elimination, but the importance is inadequately studied. Disease-induced alternations in hepatic blood flow, rates and pathways of hepatic drug metabolism and hypoalbuminemia are probably important but no systematic studies exist to clarify these points. In patients with impaired renal function, the necessity to dialyze such patients with drugs eliminated by the kidneys, for instance barbital and salicylate, is important. Since salicylate rate of metabolism is slowed at high concentration,[6] *i.e.* > 1000 mg/liter due to saturation of glycine conjugating pathways, early hemodialysis is indicated in such overdoses.

Tolerance is another reason why serum concentrations of psychoactive drugs and response may be inconsistently related. Acute and chronic and specific and non-specific cross tolerance occur with many psychoactive drugs but at present it is not possible to translate our laboratory demonstrations of these processes into clinical use.

PATTERNS OF ACUTE DRUG INGESTION

The pattern of drug overdose occurring in three Toronto teaching hospitals, as determined by direct chemical analysis by the In-Common Toxicology Laboratory of the Clinical Institute, Addiction Research Foundation on patient gastric contents, blood or urine samples is shown in Table II. During the period of analysis (October 1972 to June 1973) the laboratory determined all commonly occurring hypnotic-sedatives, analgesics and opiates but did not routinely determine amitriptyline or benzodiazepine minor tranquilizers, such as chlordiazepoxide or diazepam. Barbiturates alone or in combination with other drugs accounted for the majority of poisonings. Alcohol was present in 25% of samples. Of all the positive samples, 25% involved more than one drug. In a study performed at the Montreal General Hospital benzodiazepine tranquilizers were the most commonly ingested psychoactive agents in overdose by patient report.[7,8] In our patients, 25% of patients on history claimed to have taken a benzodiazepine derivative. If this is representative then the incidence of multiple drug ingestion is likely between 40 and 50% of all acute drug ingestions.

TABLE II

ANALYSIS OF DRUGS*

Total Number of Patients		752
Drugs not detected		286
Drugs detected		466
Barbiturates –		
Total alone or in combination		218
Single barbiturate	108	
Barbiturate combination	71	
Barbiturate + ethanol	21	
Barbiturate + other drugs	18	
Ethanol		92
Sedative-hypnotics		74
Miscellaneous		44
Salicylate		28
Salicylate + ethanol		5
Opiates or amphetamines		5

*October 1972 – June 1973

In epidemiologic evaluation of acute drug ingestion patterns, direct analysis of gastric contents, blood or urine done by laboratories which can determine all commonly prescribed drugs will be preferable to studies which rely on reports from patients or friends. Inquiry studies tend to underestimate the incidence of ethanol and barbiturate associated overdoses and multiple drug ingestions. Laboratories which plan comprehensive epidemiologic studies must be able to routinely determine on all samples barbiturates, ethanol, benzodiazepines, nortriptyline, amitriptyline, the commonly prescribed and over-the-counter analgesics in addition to salicylates, bromides, etc. Such assays must be specific for the active form(s) of the drug and sensitive enough to detect typically occurring concentrations. Numerous hospital laboratories still rely on assays for barbiturates which can not differentiate between different barbiturates nor differentiate barbiturates from diphenylhydantoin. Chlordiazepoxide has two active metabolites[9] and in amitriptyline overdoses both amitriptyline and nortriptyline, an active metabolite, must be measured.

Stringent quality control is important in the analyses of psychoactive drugs. Table III summarizes results by various classes of laboratories identifying and quantitating an "unknown" sample containing phenobarbital prepared by the Centre for Disease Control. The range of results obtained is astonishing.

TABLE III

TOXICOLOGIC QUALITY CONTROL

Phenobarbital – Target value 20 mg/100 ml

Lab Group	**Mean***	**n**	**Range**
Public Health (11)†	15.17	11	6.1 – 26.9
Forensic (8)	17.22	8	3.2 – 32.7
Private (25)	14.47	25	1.6 – 23.6
Hospital (34)	16.41	34	2.7 – 30.0
Total	15.69	78	

More than one barbiturate was found by 7 laboratories.
*Means determined by laboratory groups
†Number of laboratories in each group

Serum Concentrations and Drug Interactions

The range and distribution of serum secobarbital and phenobarbital after acute ingestion are shown in Figures 2 and 3. Equal numbers of patients were fully conscious above and below the minimal toxic drug concentrations. Of considerable importance in appreciating the reasons for an inconsistent relationship of serum concentrations and clinical response in acute drug ingestion is the high incidence of multiple drug ingestion. The concurrent ingestion of two or more agents can result in the antagonism of expected clinical effect or enhancement of such effect. Antagonism is not surprising with combinations of drugs such as phenobarbital and amphetamines, or morphine and naloxone. Concurrent ingestion of combinations of this sort will completely alter the expected relationship of serum concentration to response. This can be particularly confusing clinically when only one of the drugs has been detected. In February 1972 an 18 month old male patient with a blood phenobarbital of 95 mg/liter was assessed who was fully conscious. Subsequent drug analysis on urine indicated the presence of amphetamines.

The frequency distribution of initial blood ethanol concentrations and levels of consciousness in 95 patients after acute drug ingestion is shown in Figure 4. Physicians assigned a global rating of level of consciousness on the basis of I: fully awake, II: lethargic-drowsy, III: obtunded but arousable, IV: stuporous but responds to pain, V: coma without response to pain. Other psychoactive drugs concurrently ingested included barbiturates, glutethimide, methyprylon, amitriptyline and benzodiazepines. In subjects ingesting alcohol alone, as blood ethanol concentration increases, the level of consciousness decreases. Coma appears at blood ethanol concentration > 5000 mg per liter. In the presence of other psychoactive drugs the mean levels of consciousness are significantly deeper for each ethanol interval. Coma is associated with blood ethanol concentrations of approximately 3500 mg per liter. These results indicate that other psychoactive drugs enhance ethanol's pharmacologic effect in decreasing the level of consciousness.

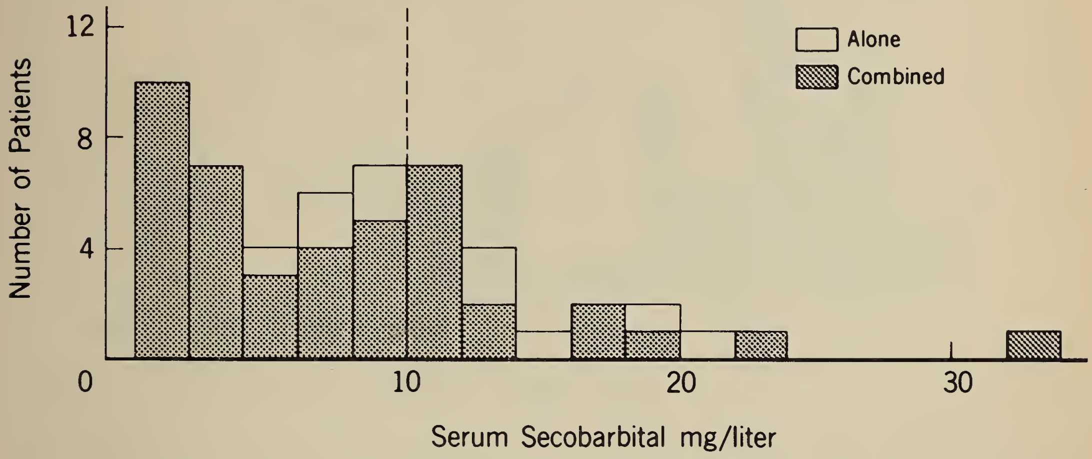

Figure 2. *Distribution of Serum Secobarbital Concentrations in 51 Patients after Acute Ingestion. February – August 1973. The vertical dashed bar indicates the usual minimal toxic concentration.*

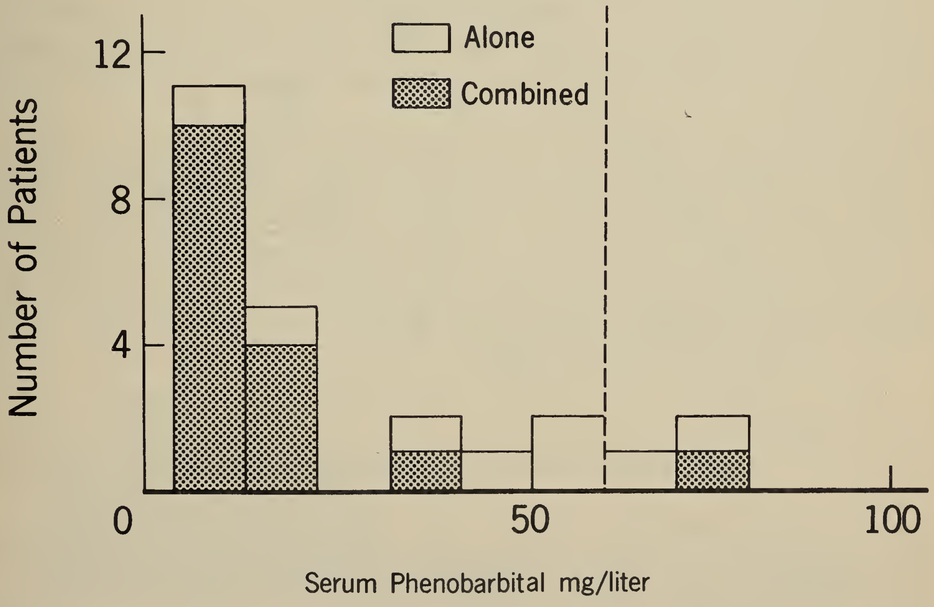

Figure 3. *Distribution of Serum Phenobarbital Concentrations in 24 Patients after Acute Ingestion. February – August 1973. The vertical dashed bar indicates the usual minimal toxic concentration.*

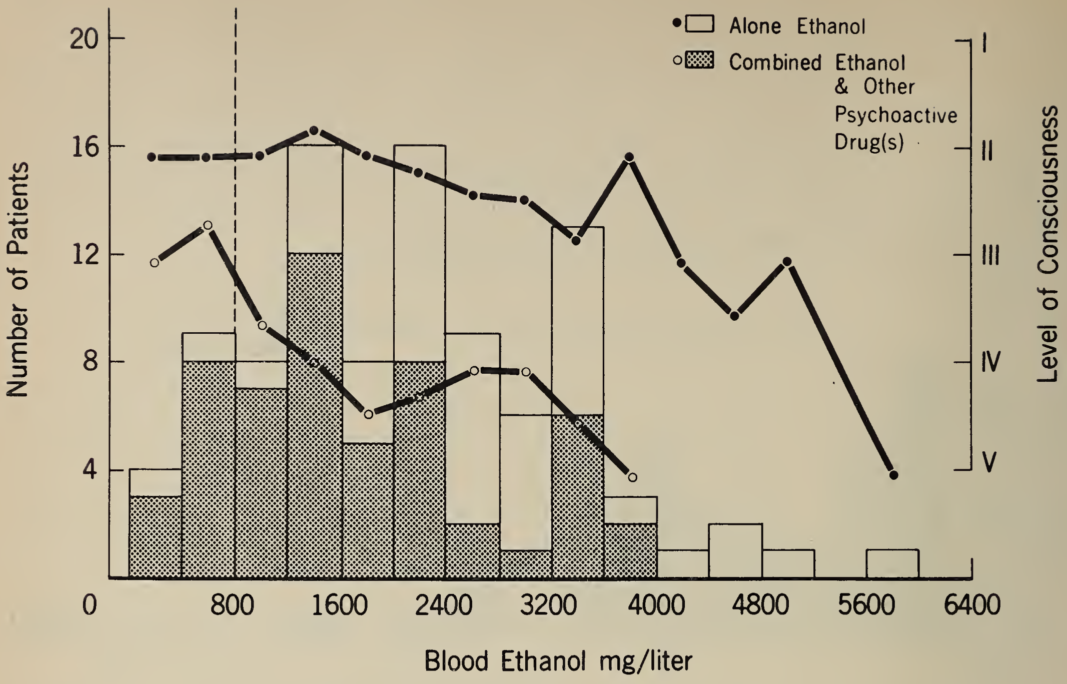

Figure 4. *Distribution of Blood Ethanol Concentrations and Levels of Consciousness in 95 Patients after Acute Drug Ingestion. February – August 1973. The vertical bar indicates the legal standard of intoxication in Ontario.*

A 30 to 50% lower barbiturate concentration is lethal when associated with concurrent ethanol ingestion.[10] Similar potentiation can be shown for other drugs. Methyprylon and salicylates ingested concurrently with other psychoactive drugs will for any given serum concentration be associated with deeper levels of consciousness. (Figures 5 and 6).

Since salicylate has little direct effect on level of consciousness, possibly the greater mental obtundation with concurrent ingestion reflects only the other ingested drug, and that analgesics are so frequently consumed in our society. The low concentrations of salicylate found in the majority of patients would support this conclusion. On the other hand one must be very cautious in predicting the extent, presentation or even direction of interactions between psychoactive drugs.

PHYSICIANS' USE OF TOXICOLOGY RESULTS

Results of a retrospective chart review to determine how drug concentration measurements of psychoactive drugs were utilized by physicians in the management of patients who had acutely ingested drugs is summarized in Table IV. Patients included were seen in four University of Toronto teaching hospitals between November 1971 and June 1972. All patients who had samples of gastric contents, blood or urine sent to the In-Common Toxicologic Laboratory of the Clinical Institute, Addiction Research Foundation were included if the analysis was asked for on an emergency or "stat" basis.

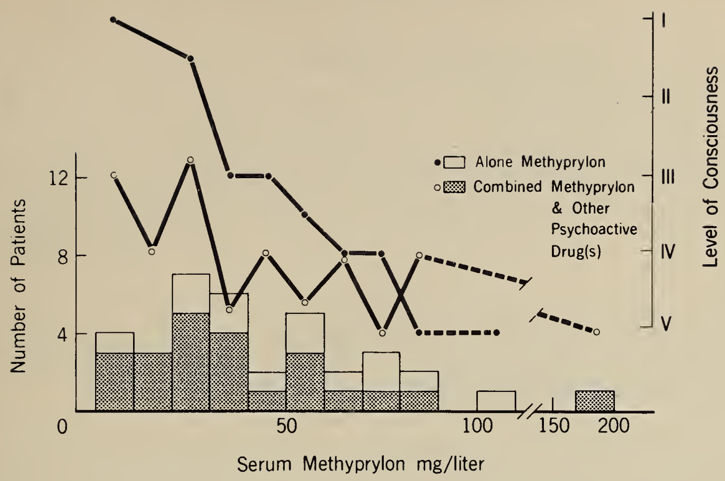

Figure 5. *Distribution of Serum Methyprylon Concentrations and Levels of Consciousness in 36 Patients after Acute Ingestion. February – August 1973.*

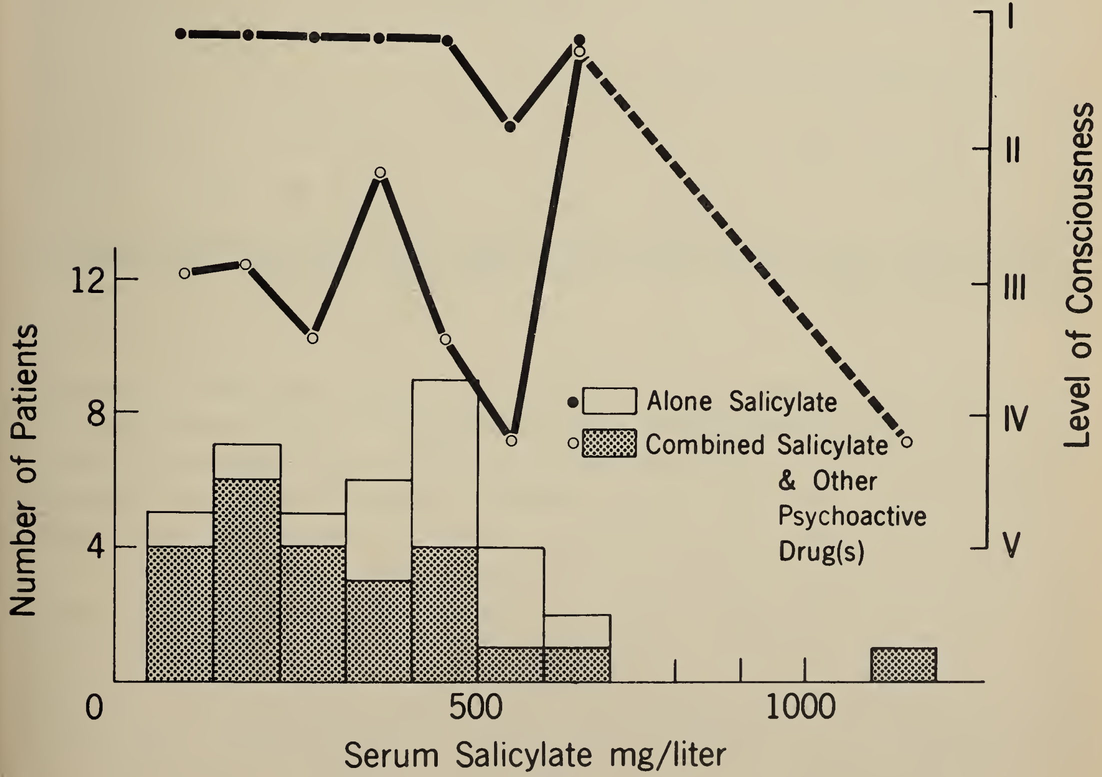

Figure 6. *Distribution of Serum Salicylate Concentrations and Levels of Consciousness in 39 Patients after Acute Drug Ingestion. February – August 1973.*

TABLE IV

CLINICAL USE OF DRUG ANALYSIS*

Clinical Indication for Emergency Drug Analysis	
Suspected Acute Ingestion	62
Suspected Chronic Drug Use	30
Not Specified	17
Total:	109
Influence of Drug Analysis on Patient Management	
Management specifically modified	12
Management confirmed as appropriate	22
No change in management	75
Total:	109
Physician Awareness of Drug Analysis Results	
Results discussed in physicians' notes	14
Results recorded in physicians' or nurses' notes	20
Report in laboratory section	50
Result not in chart	25
Total:	109

*Composite results of 4 Toronto teaching hospitals, November 1971 – June 1972. Includes emergency requests for drug analysis in blood. A total of 122 patients had analyses performed. Multiple determinations were counted as a single request.

Eleven charts could not be located. No drug was detected in 53% of patients. Charts were searched for indications of why analysis was requested. Despite the request being for "acute overdose", 44% of patients were non-emergency problems. In 22.9% of the charts, the results were not available although the results are routinely phoned to the supervising physician; in 45.8% results were found only in the laboratory section. In 31.3% a specific note of the results was made by the physician or nurse. The impact of the concentration determination on patient management was determined by examining the time of receipt of results, changes in physicians' orders and time of patient discharge from hospital. In 70% of patients, patient management was not altered. Typical patients in this group were discharged from Emergency before the results were available. The interpretation of these findings must be cautious. The fact that there was no change in therapy does not necessarily mean that the physician had not made use in some intangible way of the analytical results such as assuring himself that the problem was not a drug related one. These results focus attention on the limits of usefulness of the concentration determinations of psychoactive drugs for the physician. Limitation of usefulness and lack of concern about the meaning of these concentrations by physicians is not surprising. The relationship of dose, serum concentration and effect is highly variable. Many overdoses are mild and require no therapy. For the severe toxicity of psychoactive drugs there are no specific antidotes or specific treatments. Support of ventilation, circulation and renal function is the typical and preferred management.[8, 11] Improvement in hospital facilities and equipment for support of such vital functions has diminished the mortality of drug overdose to less than 4.7% in patients requiring hospitalization.[8]

GUIDELINES FOR DETERMINING SERUM CONCENTRATIONS AFTER ACUTE DRUG INGESTION

The majority of acute drug ingestions are mild and require no treatment. Determination of serum drug concentrations is not necessary for medical reasons in such fully conscious and asymptomatic patients. Nor will they contribute to patient management when a patient responds to specific therapy, for instance uncomplicated heroin, morphine or other opioid-induced respiratory depression, which is effectively and dramatically reversed with naloxone.

In patients who require hospitalization or observation after alleged accidental or suicidal drug ingestion, identification alone of the drug(s) in blood, urine or gastric content present will usually *initially* be adequate and often helpful. (Table V) This is particularly important for drugs where specific therapy is available e.g. amitriptyline, methanol, salicylate. The majority of hospitals should be able to identify the most commonly encountered psychoactive drugs, ethanol, barbiturates and salicylates. It is unrealistic to expect or to develop specialized, expensive comprehensive analytic services in each hospital. Regional or in-common laboratory facilities should be developed and should have full capability to rapidly identify hallucinogens, benzodiazepines, amitriptyline, nortriptyline, amphetamines, phencyclidine, phenothiazines, and MAO inhibitors, etc. Overdose with some of these drugs is rare but when encountered poses such a puzzling clinical picture that establishment of diagnosis is important to clarify the clinical situation. During the past two months seven patients have been admitted to Toronto hospitals with diagnoses of "hysteria" and "seizures", or mescaline overdose. These patients have been found to have phencyclidine in their blood, clarifying the diagnosis and management. Disappearance of the drug from the blood was associated with clinical improvement.[12]

The systematic epidemiologic studies of drug overdose are needed and should be done in conjunction with laboratories with full analytical facilities and physicians interested in the management of these patients.

There are a few indications for quantitation of psychoactive drugs. (Table V) In the patient who presents comatose due to whatever cause, it is important to determine whether drugs are present in high enough concentrations to be compatible with coma. Decisions to dialyze patients who have ingested salicylates, methanol or barbiturates should be made as early as possible in the clinical course. Quantitation of drugs may also be helpful in the overdose patient who is clinically deteriorating since

TABLE V

INDICATIONS FOR DRUG DETERMINATION AFTER ACUTE DRUG INGESTION

Indications for Identification Only

1. Clinical care of patients
    a) initial evaluation of suspected acute ingestion
    b) suspected chronic drug misuse
2. Legal

Indications for Quantitation

1. Clinical care of patients
    a) patient comatosed on first examination
    b) drug serum concentration proven to have prognostic significance or indicate form of therapy to be instituted, e.g. salicylate, methanol
    c) complex or deteriorating clinical situation
    d) regulation of specific therapy
    e.g. ethanol treatment of methanol poisoning
    e) following the effectiveness of treatment
    e.g. dialysis
2. Research
3. Teaching
4. Legal

serum concentrations may be slowly rising due to a large amount of drug still in the gastrointestinal tract. In the complex clinical situation of renal or hepatic failure, quantitation may clarify the clinical situation. Few systematic and comprehensive studies have been conducted to determine the potential usefulness of serum drug concentration determinations after acute drug ingestion. There is still need to further reduce the morbidity and mortality due to acute drug ingestion; to develop clinically useful measures of psychoactive drug effect; to define more exactly the relation of concentration and response in serious overdose; to quantitate the importance of inter-individual variations in elimination rates of psychoactive drugs; to determine the frequency and importance of multiple drug ingestion and drug interactions and to study alternate forms of therapy. Detection of psychoactive drugs in gastric contents, serum or urine has value in establishing a diagnosis but serves at present only as a limited guide to effective therapy. Drug concentration determinations of psychoactive drugs do not substitute for careful medical observations and

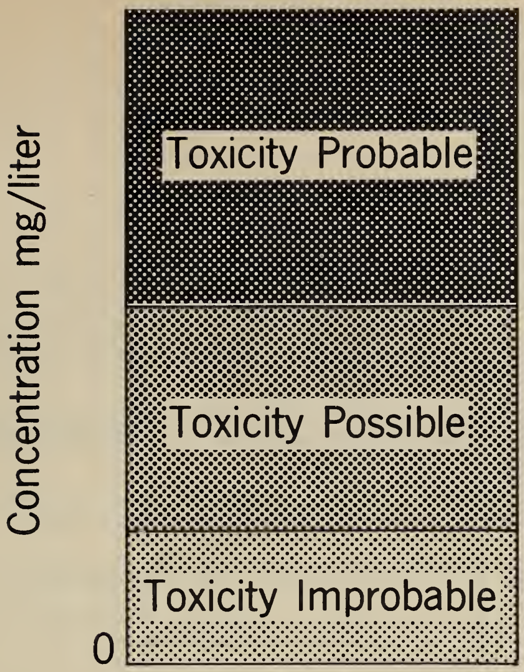

Figure 7. *Relation of Drug Concentrations and Toxicity.*

judgment and must be interpreted in the context of all clinical data. The following factors should be considered in such interpretation. The effect of a given drug concentration is altered by many physiological and pathological factors. Tolerance develops to many psychoactive drugs. A single concentration can not be interpreted if the time of ingestion is not known. The assay may not be specific or sensitive. Undetected active drug metabolites may exist. Multiple drug ingestion is encountered in 40-50% of overdose patients.

In clinical medicine there is a tradition to classify into categories. This tradition is exemplified by lists of differential diagnoses, and the problem-oriented record. Much clinical research is designed to answer whether a given manoeuvre produces a result different from the control group. Usually little attention is paid to the confidence limits of a decision; to the individual patient characteristics which influence outcome; to the kinetics of drug metabolism and its relation to toxicity. As a result, the clinician is unable for a particular patient to estimate the probability that this patient may respond, or may develop an adverse effect of therapy. Definitions of "toxic" serum concentration are an attempt to categorize. There are no discrete borders between toxic and nontoxic for most psychoactive drugs. (Figure 7) Utilizing the techniques of probit analysis it may be possible to gain clinically useful estimates for a particular patient of the possibility of a given concentration or combinations of concentrations accounting for the clinical picture.

ACKNOWLEDGEMENT

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Blood Levels of Benzodiazepines: Applications in Medicine and Toxicology

David J. Greenblatt M.D. and Richard R. Shader M.D.

ABSTRACT Reliable methods for determining blood concentrations of benzodiazepine tranquillizers and hypnotics are tedious, expensive, and beyond the means of most laboratories. For this reason, their pharmacokinetic properties and the relation of blood concentrations to clinical effects have received relatively little study. Benzodiazepine pharmacokinetics vary widely between individuals. Chlordiazepoxide and diazepam are slowly metabolized, and are biotransformed to pharmacologically active metabolites. Repeated administration causes significant drug accumulation. Cumulative effects also occur with repeated nightly use of nitrazepam. Oxazepam and lorazepam, both having 3-hydroxyl substitutions, are more rapidly biotransformed to inactive products. Accumulation of these drugs is much less important. The pharmacokinetics of other benzodiazepines are less well studied. Benzodiazepine overdosage is relatively innocuous. Very high doses and blood concentrations can be tolerated with no serious cardiovascular or respiratory depression. Occasionally, large doses of diazepam given to toxemic mothers produce prolonged neonatal depression after delivery. It appears that diazepam readily crosses the placenta, and is slowly metabolized by the immature liver.

Benzodiazepine tranquillizers first became available for clinical use in 1960. Since this time, the extent of their use has increased at an astounding pace. Currently benzodiazepines are among the most commonly prescribed drugs in the world, and their use becomes more widespread yearly.[1-6] In 1972, more than 80 million prescriptions for benzodiazepine derivatives were filled in American retail pharmacies at a total expenditure exceeding 200 million dollars.[6] Diazepam (Valium) accounted for two-thirds of these prescriptions. Figure 1 shows the structural formulas of benzodiazepines currently in clinical use or in the late stages of testing in various parts of the world. In the United States four of these drugs (chlordiazepoxide, diazepam, oxazepam, chlorzepate) are marketed as antianxiety agents, and one (flurazepam) is available as a hypnotic.

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For a number of pharmacologic agents (*i.e.*, digoxin, diphenylhydantoin, procainamide, quinidine, lithium), blood concentration determinations are increasingly recognized and used as valuable guides to rational therapeutics.[7] This is not true for the benzodiazepines, despite the prevalence of their use. These drugs are usually given on an arbitrary dosage schedule, with adjustments made on a trial-and-error basis according to vaguely-defined clinical effects. There are very few studies which relate benzodiazepine blood levels to clinical effects. Moreover, our knowledge of the human pharmacokinetics of benzodiazepines is based upon trials with relatively few subjects, performed mainly by the pharmaceutical firms that manufacture the drugs.

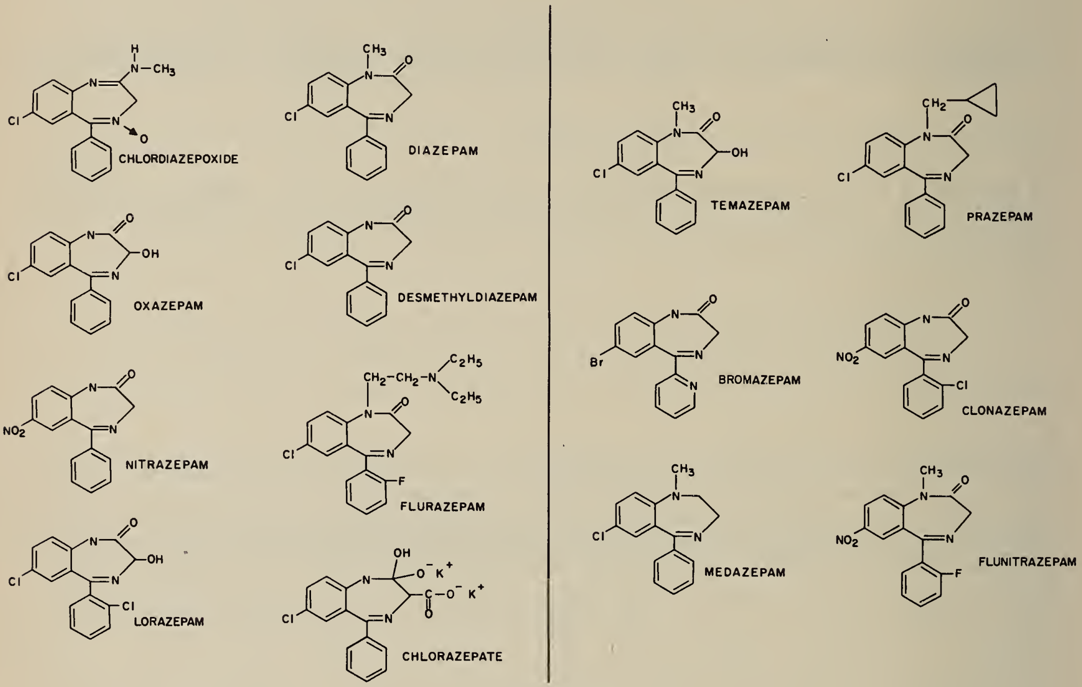

Figure 1. *Structural formulas of selected benzodiazepine tranquillizers and hypnotics.*

PROBLEMS AND OBSTACLES IN BLOOD CONCENTRATION STUDIES

There are good reasons for the relative paucity of pharmacokinetic data. Because of extensive biotransformation and/or extensive tissue distribution, benzodiazepines are present in body fluids only in trace amounts after usual therapeutic doses. Adequate quantitation requires sophisticated, expensive analytic methods which are beyond the means of most laboratories. In many analytical systems, the major metabolic products of benzodiazepines administered in therapeutic doses cannot be distinguished from the parent compounds. Furthermore, the psychopharmacologic activities of the metabolites themselves often mimic those of the precursor drugs in intensity and duration of action. Finally, in some methods, the benzodiazepines may undergo chemical alteration in the process of analysis.

Gas-liquid chromatography (GLC) is currently considered the most useful approach to quantitation of benzodiazepines in body fluids. However, numerous problems have been encountered. At high column temperatures, 3-hydroxy and N-oxide derivatives, in particular chlordiazepoxide, demoxepam, and oxazepam, become thermolyzed to form benzophenones or rearrange to form quinazolines.[8, 9] Only recently have GLC techniques been adapted for determinations of these benzodiazepines. Other analogues (diazepam, temazepam, desmethyldiazepam, medazepam) are thermally stable, but assay of the intact drugs may not yield optimal sensitivity.[10-13] Acid hydrolysis of the benzodiazepines to form benzophenones, together with use of electron-capture detection, increases the sensitivity of the procedure considerably. However, the preparatory hydrolysis is time-consuming, and some specificity is lost in the process. Diazepam and temazepam, for example, produce the same hydrolysis product (2-methamino-5-chloro-benzophenone MACB); similarly, desmethyldiazepam and oxazepam, when hydrolyzed, yield 2-amino-5-chlorobenzophenone (ACB) (Fig. 2). Recently a radioimmunoassay technique has been developed for diazepam and its major metabolite.[14] This technique is much less expensive and tedious than GLC. It is likely that the availability of methods such as radioimmunoassay will facilitate future study of benzodiazepine pharmacokinetics and correlations of body fluid concentrations with clinical effects.

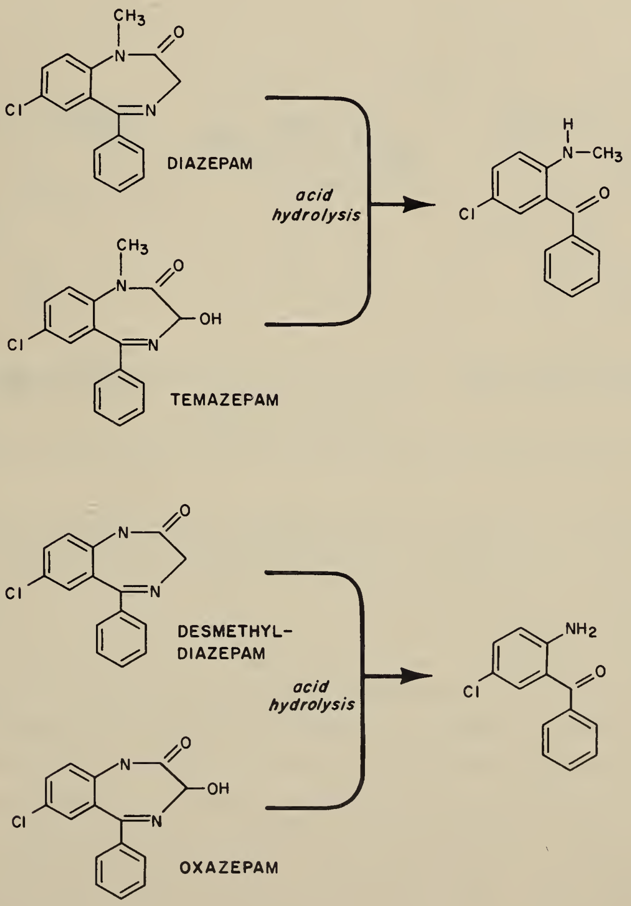

Figure 2. *Acid hydrolysis products of diazepam and its metabolites. (Reprinted with permission*[15]*)*

CLINICAL USE OF BENZODIAZEPINE BLOOD CONCENTRATIONS

Specific principles and problems in benzodiazepine blood concentration assays, and the use of these techniques in clinical studies, are considered in this section. This review is limited to investigations involving humans. Reviews of animal and *in vitro* studies are available elsewhere.[15-19]

Chlordiazepoxide

Pure chlordiazepoxide (CDX) exists as a water-soluble crystalline hydrochloride salt. Studies using dog plasma suggest that it is 87-88% protein-bound over a wide range of concentrations.[20] When exposed to ultraviolet light the N-oxide of CDX isomerizes to form a cyclic oxaziridine (Fig. 3).[21] The isomer has a high melting point and different pharmacologic properties. This accounts for the manufacturer's recommendation that only freshly-prepared solutions be used for parenteral injection.

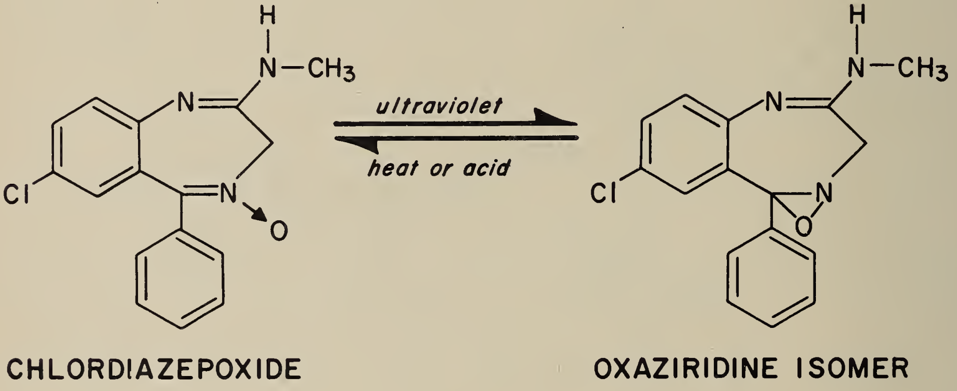

Figure 3. *Photoisomerization of chlordiazepoxide. (Reprinted with permission[15])*

Early quantitative assays for chlordiazepoxide did not distinguish the parent drug from its major metabolites, desmethylchlordiazepoxide (DMCDX) and demoxepam (DMX). Smyth and associates[22, 23] in 1963 described a quantitative spectrophotometric assay using the products of acid hydrolysis. Administration of a single large oral dose (100 mg) of chlordiazepoxide to nine volunteers resulted in average maximum *total* drug concentrations (chlordiazepoxide plus metabolites) of 5 μg per ml five hours following ingestion.[23] Blood levels fell slowly, and less than 2% of the total dose was excreted unchanged in the urine in the next 48 hours. Koechlin and D'Arconte,[24] also in 1963, reported a more specific spectrofluorometric micro method sensitive to blood levels as low as 0.25 μg per ml. This assay distinguished chlordiazepoxide from demoxepam, the ultimate metabolite, but not from desmethylchlordiazepoxide, the intermediate metabolite. The half-life of chlordiazepoxide in humans (after a single oral dose of 30 mg) was estimated to be 20 to 24 hr. Studies using ^{14}C-labeled chlordiazepoxide yielded similar results.[25] Demoxepam was the major metabolite detected in man, but DMCDX was not identified as an intermediate. Because of the non-specificity of these assays, the half-life of chlordiazepoxide *per se* was overestimated. This error was not clinically apparent, however, since both DMCDX and DMX have psychopharmacologic activity approaching that of chlordiazepoxide itself.[15]

In 1966, Schwartz and Postma,[26] adapting the method of Koechlin and D'Arconte,[24] first identified desmethylchlordiazepoxide as a metabolite of CDX. After a single intravenous dose of 100 mg in humans, CDX alone had a half-life of 14 hr, and both DMCDX and DMX were identified in the blood. DMCDX was then postulated as a metabolic intermediate in humans. In subsequent studies, Schwartz and associates[27] elucidated the metabolic pathway of chlordiazepoxide which is currently understood as correct (Fig. 4). A 20 mg oral dose in six human subjects produced a maximum blood level of 0.78 to 1.24 μg per ml from 2 to 6 hours after the dose. CDX levels then declined slowly, but with a highly variable half-life ranging between individuals from 6.6 to 28 hours. Desmethylchlordiazepoxide was detected in plasma at levels as high as 0.46 μg per ml anywhere from 8 to 24 hours after the dose. Demoxepam appeared even later and at lower levels. Demoxepam is slowly excreted, and appears unchanged in the urine together with a variety of hydroxylated and glucuronide-conjugated metabolites.[28] Figure 5 shows blood concentrations of CDX and DMCDX for the first 24 hours following 25 mg oral doses of CDX to two healthy young female subjects in our laboratory. These results emphasize the interindividual variations in chlordiazepoxide pharmacokinetics. Generalizations from studies involving small numbers of subjects can be made only with caution.

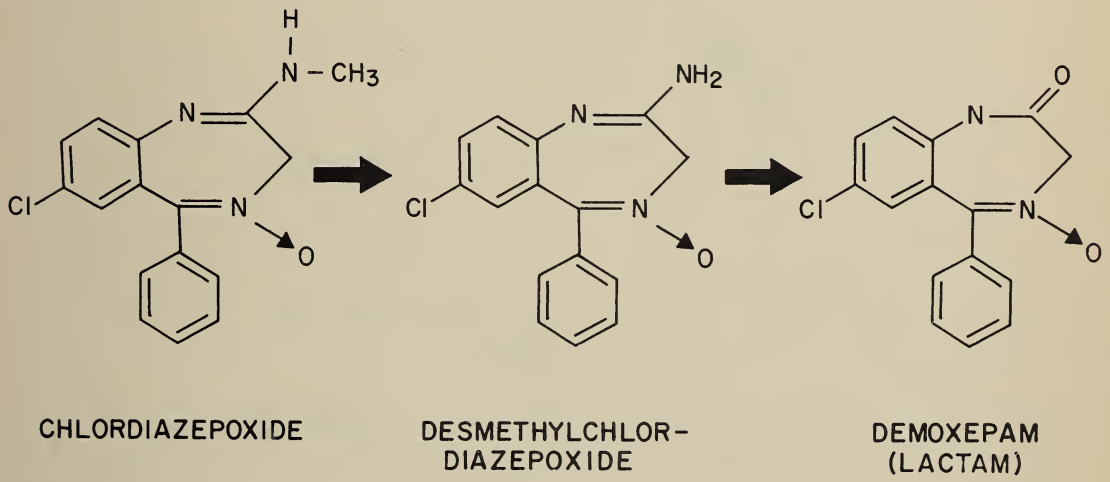

Figure 4. *Metabolic disposition of chlordiazepoxide in man.*

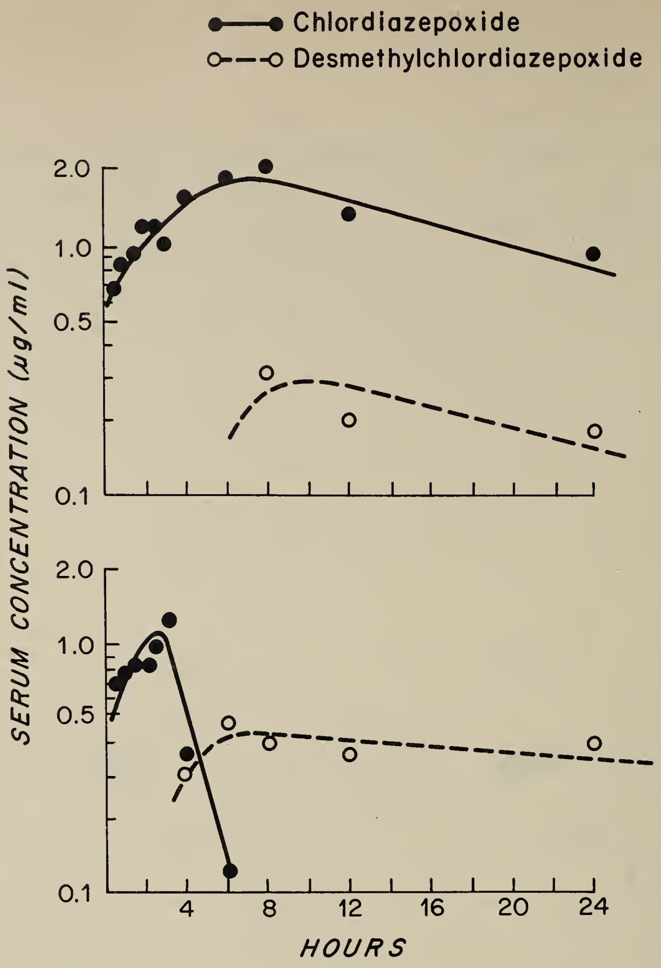

Figure 5. *Two healthy female volunteers received single oral doses of chlordiazepoxide (25 mg) at time zero. Blood concentrations of chlordiazepoxide and desmethylchlordiazepoxide are shown for each subject for the first 24 hours after the dose. No demoxepam was detected.*

Gottschalk and associates[29, 30] investigated the relation between blood concentrations of CDX and antianxiety effects in 18 subjects. Following a single oral dose of 25 mg, blood levels ranged from 0.26 to 1.63 μg per ml after 1½ to 2½ hours, with a mean of about 0.86 μg per ml. There appeared to be no correlation of blood level and body weight. Antianxiety effects, as determined by speech content analysis, were most evident among individuals with blood concentrations of at least 0.7 μg per ml. Unfortunately, it is not clear whether the time of blood sampling corresponded with peak blood concentrations.

Dr. Ina Zingales of Cleveland State Hospital described a specific GLC assay for chlordiazepoxide which is sensitive at low levels.[31] The drug is extracted from plasma into 1.5% isoamyl alcohol in n-heptane. The extract is injected directly into the chromatograph. Presumably, the extent of pyrolysis is reproducible, allowing reliable quantitation. Single oral doses of 5 mg in two volunteers produced blood concentrations of 0.18 μg per ml two hours later; after 10 mg, the level was 0.4 μg per ml. After chronic therapy with total daily doses of 75 to 150 mg, blood levels were found to range from 3.2 to 6.9 μg per ml. The reliability of this GLC method has not yet been confirmed by other groups.

Diazepam

Diazepam is lipid soluble and relatively water insoluble, and is strongly bound to plasma protein (95%) over a wide range of concentrations.[20, 32] DeSilva and associates[33] described the first GLC assay for diazepam in 1964. This method required acid hydrolysis prior to chromatography. DeSilva and Puglisi[34] subsequently reported a method for GLC of intact diazepam using electron capture detection. This method is adequately sensitive, and can quantitate diazepam and all of its metabolites in body fluids with no loss of specificity. Without electron capture, GLC of intact diazepam is inadequate except in cases of poisoning or overdosage.[35]

The metabolism of diazepam in man has been studied by a number of groups and their results are in general agreement.[32-34, 36-39] Diazepam is rapidly absorbed after oral administration. After a single oral dose of 10 to 15 mg, peak levels in the range of 0.2 to 0.3 μg per ml occur within two hours. There is wide variation between individuals. Most subjects are drowsy when blood concentrations are in this range. Blood levels fall first rapidly, then slowly. The half-life during the initial distribution phase is less than 2 hours. The drug is then slowly biotransformed and excreted, with a half-life ranging between individuals from 20 to 50 hours. This biphasic elimination pattern is most marked after intravenous administration. A 10 to 20 mg dose produces a level at 4 min of about 1 μg per ml, coincident with profound clinical sedation and a short period of anterograde amnesia. Blood concentrations rapidly fall to about 0.25 μg per ml within an hour, accompanied by clinical "recovery." The rate of elimination then slows. Baird and Hailey[39] have noted a "resurgence" of blood levels at approximately six hours following the dose, at which time subjects again become drowsy. This phenomenon may be related either to mobilization of diazepam from lipid storage sites, or to release of diazepam or its active metabolites from the enterohepatic circulation. A similar phenomenon was noted after intramuscular (IM) diazepam.[40] The initial phase of drug absorption after IM injection appears to be erratic and irregular.[40] It is not known whether the bioavailability of IM diazepam is equivalent to that of intravenous. Like diazepam, injectable preparations of digoxin and diphenylhydantoin utilize a propylene glycol solvent vehicle; neither of these drugs is completely absorbed from IM injection sites.[41-43] Four-fold elevations in serum creatine phosphokinase (CPK) have been noted after IM diazepam,[44] suggesting that diazepam should not be given by this route when serum CPK is of diagnostic importance.

Repeated oral administration of diazepam in man leads to accumulation. If the drug is given daily in divided doses, blood levels gradually rise until a plateau is reached after 5 to 7 days of therapy. The usual plateau concentrations are in the range of 0.1 to 0.4 μg per ml, depending on the size and frequency of the doses. Pharmacologically active metabolites, temazepam (TMZ) and desmethyldiazepam (DMDZ), are detectable in the blood following a single dose.[39] After several days of therapy, desmethyldiazepam, the major metabolite in man, also accumulates. At equilibrium, levels of DMDZ are close to those of DZ.[38] In one report,[45] steady-state DMDZ concentrations exceeded those of DZ in a patient receiving phenobarbital concurrently; it is possible that enzyme induction was responsible for this reversal.

When therapy is terminated, concentrations of diazepam and its metabolite decline, but DMDZ, having an apparent half-life as long as 96 hours in some subjects, persists in the plasma longer than diazepam. The metabolic pathway of diazepam in humans is shown in Figure 6. Oxazepam glucuronide is the major urinary metabolite. Conjugated derivatives of desmethyldiazepam are also detected in the urine.

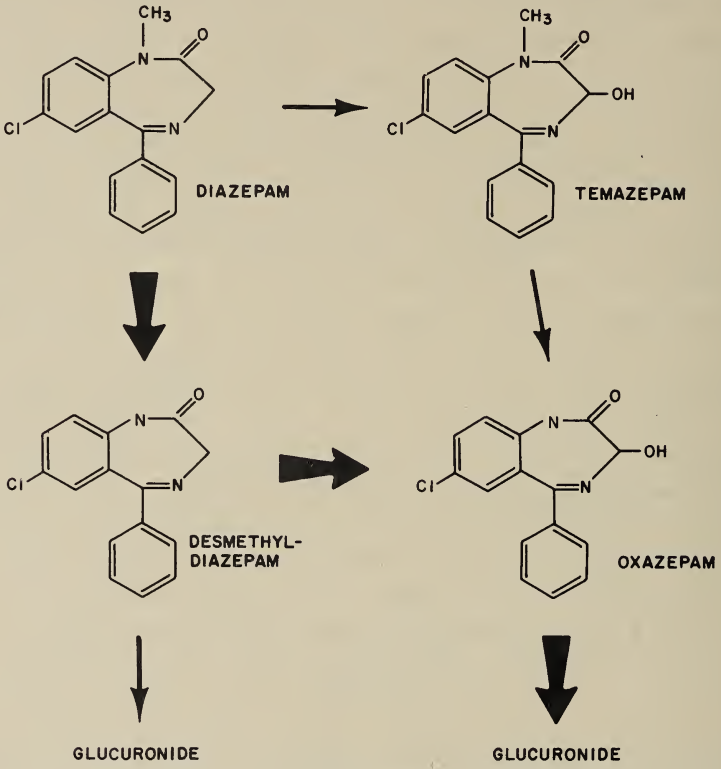

Figure 6. *Metabolic disposition of diazepam in man. Major pathways are shown by heavy arrows.*

Studies correlating diazepam concentrations with clinical effects are few in number. Booker and Celesia[46] made some preliminary observations relating peak diazepam concentrations after intravenous injection to suppression of epileptiform discharges in patients with seizure disorders. Peak blood concentrations of 0.6 μg per ml or greater were associated with suppression of discharges, but concentrations at which they recurred were greatly variable. Further study is needed to elucidate the nature of this correlation.

Oxazepam and Lorazepam

Both of these derivatives have 3-hydroxy substitutions, allowing easy metabolic conversion directly to inactive conjugates. Accumulation of these drugs during chronic therapy is much less important than with chlordiazepoxide or diazepam. This may account for reports in several controlled clinical trials that long-term treatment with oxazepam and lorazepam produces drowsiness less often than diazepam.[15]

Oxazepam and lorazepam can be reliably quantitated by GLC of the benzophenones formed by acid hydrolysis.[47-50] In humans a single 45 mg oral dose of oxazepam produces maximal blood concentrations of the free drug in the range of 1.1 μg per ml between one and four hours after administration. The serum half-life of oxazepam ranges from 3 to 21 hours in different individuals; in one study the mean half-life was 4 hours,[47] and in another the mean was 15 hours.[48] Oxazepam glucuronide, the major metabolite, rapidly appears in the blood. Eighty percent of the total dose is excreted in the urine after 72 hours; 10% or less appears in the feces.[51, 52] Oxazepam glucuronide is also the major urinary metabolite. Large oral doses of lorazepam (7.5 mg) produce free drug concentrations barely exceeding 0.05 μg per ml despite profound sedative effects.[50] Concentrations fall slowly over 48 hours. Lorazepam glucuronide is detectable in the blood within a few hours, and reaches maximum levels of about 0.1 μg per ml 4 to 12 hours after the dose. Sixty-five percent of the dose is recoverable in the urine after three days, with lorazepam glucuronide the major urinary constituent in man.[53]

Nitrazepam

Nitrazepam is not available in the United States, but is a widely used hypnotic agent in Britain and Europe.

Some groups have used GLC for determination of blood concentrations of nitrazepam.[54] The intact drug can be assayed directly, with column temperatures and carrier gas flow rates higher than those used in chromatography of other benzodiazepines. The sensitivity and reliability of GLC may be considerably improved by acid hydrolysis prior to analysis. However, some specificity is lost, since the two major metabolites, formed by reduction and acetylation at the 7-position, yield the same benzophenone product after hydrolysis.

Most of our knowledge of nitrazepam pharmacokinetics comes from groups using spectrophotometric assays. The technique utilizing the Bratton-Marshall reaction does not appear to be sufficiently sensitive. Sawada and Shinohara[55] estimated the plasma half-life of nitrazepam in man to be about 7 hours. Subsequent studies by Rieder and associates[56, 57] using a more reliable colorimetric method,[58] have suggested that this figure is an underestimate.

The absorption of nitrazepam following oral administration varies from 53 to 94% of the total dose. In most subjects, 10 mg taken orally produces maximum blood levels of 0.08 to 0.10 μg per ml 2 hours after ingestion. Nitrazepam is 87% protein-bound. The half-life of unchanged nitrazepam is 25 hours after oral administration and 21 hours when the drug is given intravenously. Consecutive nightly administration results in some degree of accumulation. A daily dose of 5 mg produces a steady-state blood level of about 0.04 μg per ml after 4 days of continuous therapy. Both the 7-amino and 7-acetamido derivatives (Fig. 7) also accumulate in the blood, but both of these metabolites are essentially inactive.

The disposition of nitrazepam is dependent upon the route of administration. When given orally, 65 to 71% of the dose eventually appears in the urine, and 14 to 20% appears in the feces. After intravenous dosage, 93% is recovered in the urine

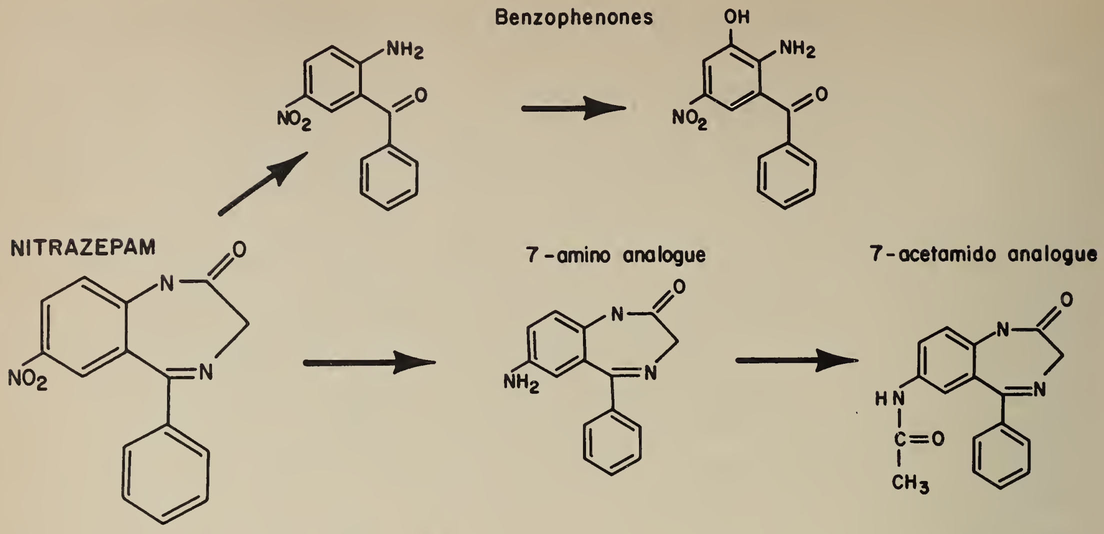

Figure 7. *Metabolic disposition of nitrazepam in man.*

and 2 to 13% in the feces. In both cases, the unchanged 7-acetamido derivative is the major metabolite. The 7-amino metabolite is quantitatively more important after intravenous administration than after oral. The two benzophenone metabolites (Fig. 7) are more important when nitrazepam is given orally, and appear predominantly as glucuronides. Metabolites formed by 3-hydroxylation are quantitatively minor.

Flurazepam

Flurazepam is metabolized and excreted so rapidly by humans that within several hours of a very large dose (90 mg), blood levels of the intact drug become barely detectable by spectrophotofluorometric assay (less than 0.01 μg per ml).[59, 60] More than 50% of the total dose appears in the urine in 24 hours, with eventual urinary excretion accounting for 80% or more of the total dose.[61] Eight to nine percent appears in the feces. Metabolism proceeds by stepwise dealkylation of the 1-diethylaminoethyl side chain, followed by oxidation of the amino group to an alcohol or carboxyl (Fig. 8). The aldehyde intermediate is postulated. In man the major urinary metabolite is the 1-hydroxyethyl analogue in conjugated form. Other metabolites are present in smaller or trace amounts. After oral flurazepam in man (90 mg), blood concentrations of the 1-hydroxyethyl and 1-unsubstituted analogues are higher than the parent drug, but even these levels do not exceed 0.1 μg per ml.

Medazepam

GLC has been adapted for sensitive and specific assays of medazepam in blood and urine.[34] An oral dose of 50 mg in man results in a brief blood level peak of about 1 μg per ml one to two hours after the dose. Concentrations rapidly fall to about 0.1 μg per ml in 2 to 3 hours, then slowly decline to 0.03 μg per ml over 24 hours. Diazepam is detectable in blood shortly after a single oral dose of medazepam, together with traces of desmethyldiazepam.[34] After several single daily doses of 50 mg, medazepam does not accumulate in the plasma to any significant degree since each dose is rapidly metabolized and eliminated. Its metabolites, however, (diazepam, desmethyldiazepam, and desmethylmedazepam) do accumulate. In man, 55 to 63% of a dose is eventually recoverable in the urine, with 7 to 22% appearing in the feces.[62] Oxazepam glucuronide is the predominant urinary metabolite.

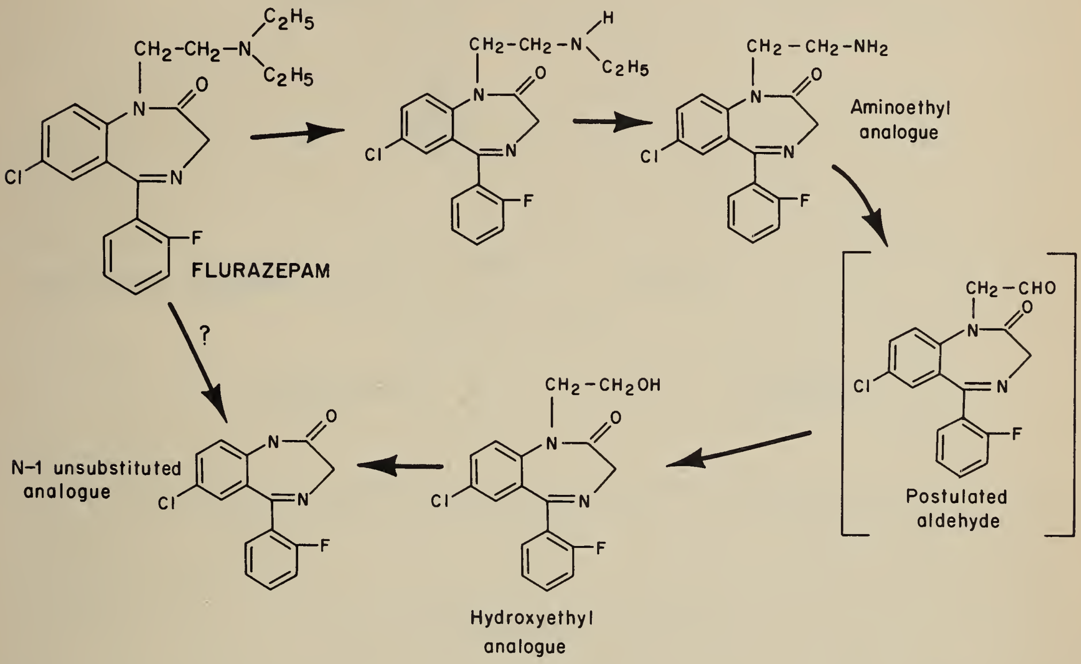

Figure 8. *Metabolic disposition of flurazepam in man.*

Prazepam

Prazepam's unusual N-1 cyclopropylmethyl substitution renders it relatively resistant to metabolic degradation. Prazepam is also absorbed more slowly than other benzodiazepines. After an oral dose in man (25 mg), blood concentrations rise very gradually over 4 to 6 hours.[63] The delayed absorption pattern of prazepam suggests a possible "hang-up" in the gastrointestinal tract, perhaps due to binding by mucosal cells, in addition to enterohepatic circulation.

The pharmacokinetics of prazepam have been studied by radioactive tracer investigations. The half-life of total radioactivity in human blood was found to be 43 to 57 hours in 4 of 5 subjects in one study,[64] and approximately 78 hours in another.[65] Most of the radioactivity was located in unconjugated compounds, which were eliminated more slowly than the conjugated metabolites. Fourteen percent of the total dose appeared in the urine after 24 hours and 22% in 48 hours. Fecal excretion was 7% in 48 hours. Nearly all the urinary radioactivity was found in glucuronide metabolites, primarily the glucuronides of oxazepam and 3-hydroxyprazepam. Small amounts of unchanged desmethyldiazepam also were identified. Metabolic pathways of prazepam are shown in Figure 9.

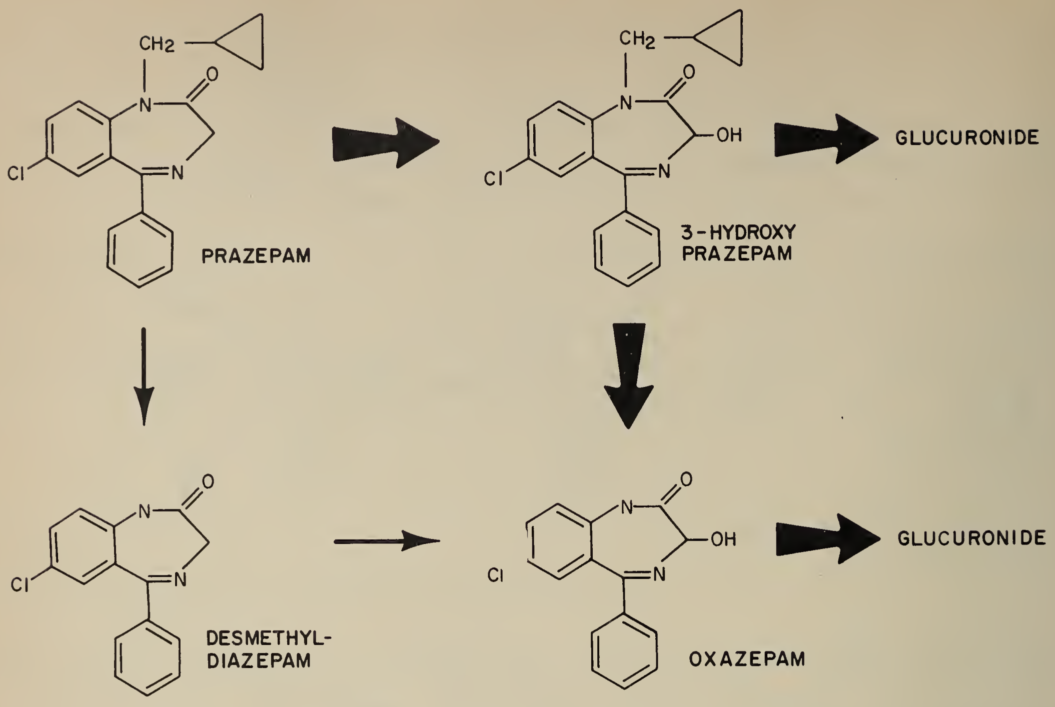

Figure 9. *Metabolic disposition of prazepam in man. Major pathways are shown by heavy arrows.*

BENZODIAZEPINE OVERDOSAGE

The problem of poisoning with psychotropic agents has been reviewed in several publications.[66-68] As tranquillizing agents become more widely used, overdosage becomes more common. Minor tranquillizers are involved in 10 to 25% of cases of intentional self-poisoning, according to several recent surveys.[69-71] Ingestion of multiple drugs is common; poisoning with barbiturates, glutethimide, or methaqualone remains the most important problem, both numerically and with respect to the gravity of the medical syndrome. Lawson and Mitchell[72] reviewed nearly 1000 cases of drug ingestion seen since 1960. About 12% involved benzodiazepines, and none of these patients died. In another review of almost 1200 cases, Sharman and associates[73] also found that benzodiazepines were involved in 12% of cases. Barbiturate overdosage accounted for most of the total cases and most of the deaths in both of these series.

Benzodiazepines are the most benign of all psychotropic drugs with respect to the danger of overdosage.[66-68] A total of 5,849 deaths due to drug ingestion are on file at the General Registrar's Office in London for the period 1968 through 1969. In only sixteen cases was a benzodiazepine by itself implicated (nitrazepam, 8 times; chlordiazepoxide and diazepam, each 4 times).[74, 75] In the medical literature there are no reported cases of fatal overdosage due to benzodiazepines alone.

Nitrazepam poisoning has been studied in detail at the Regional Poisoning Treatment Center of the Edinburgh Royal Infirmary.[76] Out of 1,176 hypnotic drug overdosages, 102 involved nitrazepam.[77] Only 6 patients were deeply comatose and none was obtunded for more than 12 hours. No patients required intubation, and only one was hypotensive. Among 706 barbiturate overdosages, however, 35% were deeply comatose, 15% remained comatose for more than 12 hours, 12% required intubation, and 24% were hypotensive. Poisoning with methaqualone and glutethimide was of equal gravity. Electroencephalographic studies[78] were consistent with deep coma in many barbiturate ingestions, but revealed predominantly beta and theta activity in nitrazepam overdosage. Tomsett[79] performed blood level studies in 10 cases of overdosage. Concentrations of unchanged nitrazepam ranged from 0.1 to 7.0 μg per ml. Metabolites formed by reduction of the 7-amino group were present in concentrations of 0.7 to 12.0 μg per ml. Unfortunately, details of the quantities of drug ingested are not available.

In three series of chlordiazepoxide overdosage, a total of 148 cases were reviewed.[80-82] Doses ranged from 60 to 2,250 mg or as high as 50 mg/kg. Patients' ages ranged from 15 months to 65 years. There was no deep coma, respiratory depression, or hypotension in any of the cases, and all recovered without sequelae. Patients had symptoms of somnolence and drowsiness, but generally were rousable. Symptoms usually disappeared within 48 hours of ingestion. Jatlow[83] analyzed blood samples of 60 patients following large overdoses of chlordiazepoxide. Blood concentrations ranged from 2.0 to 66 μg per ml. Levels greater than 20 μg per ml were associated with drowsiness; patients with concentrations of 40 μg per ml were asleep but rousable. True coma was extremely rare, even in patients with levels exceeding 60 μg per ml.

Other reports of blood concentrations in benzodiazepine overdosage are anecdotal.[84-86] Cruz and associates[87] reported a fatal case of multiple drug ingestion, involving chlordiazepoxide as well as barbiturates, ethanol, ethchlorvynol, and propoxyphene. The initial blood concentration of chlordiazepoxide was 24 μg per ml, and elimination appeared to be hastened by hemodialysis. The drug was not detectable in the dialysate, however. Both chlordiazepoxide and diazepam are strongly protein-bound, and dialysis would not be expected to be of great help. Fortunately, dialysis is seldom required. Decker *et al*[88] found that *in vitro* dialysis of plasma containing chlordiazepoxide and diazepam caused very little drug removal. Dialysis against activated charcoal, however, enhanced drug removal considerably. Rice *et al*[89] showed that forced diuresis was of little value in experimental chlordiazepoxide poisoning.

Parenteral diazepam is frequently administered as a psychosedative in association with obstetrical anesthesia and analgesia. The drug readily crosses the placenta in humans.[90-92] Numerous investigators report that diazepam concentrations in cord blood or fetal plasma exceed those in maternal blood.[90-97] The reasons for this are not clear. In usual doses, however, maternal diazepam administration is not harmful to the infant, although it occasionally causes transient and clinically minor muscular hypotonicity,[98] hypothermia,[93] or respiratory depression.[99] On the other hand, large doses of diazepam given during toxemic pregnancies can cause serious neonatal depression. In two recent reports,[100, 101] three severely toxemic mothers received more than 100 mg of intravenous diazepam prior to delivery. Their infants were hypotonic, apneic, and required assisted ventilation. Exchange transfusion was performed on one infant.[100] In the other two neonates,[101] high blood levels of diazepam (more than 4.5 μg per ml) were found, and the drug continued to be detectable for many days after delivery. The persistence of diazepam in the blood of these infants could reflect immature drug detoxification mechanisms.[102]

DISCUSSION

Therapy with benzodiazepine tranquillizers and hypnotics can be more systematic and rational when the pharmacokinetic properties of these drugs are understood. Because of their long duration of action, chlordiazepoxide and diazepam need not be given in multiple daily doses. Twice-daily dosage is sufficient. If anxiety and agitation contribute to insomnia, two-thirds to three-quarters of the total daily dose can be given at bedtime, obviating the need for another hypnotic drug. The cumulative effects of these two drugs are of considerable importance. Therapeutic and/or toxic effects might be seen only after several days of continuous treatment. It is frequently observed that intravenous diazepam "wears off" rapidly when given to treat status epilepticus. This is due to tissue distribution rather than rapid metabolism. Repeated intravenous doses will result in drug accumulation and occasionally unexpected and longlasting central nervous system depression. Oxazepam is more rapidly biotransformed to inactive metabolites, making cumulative effects less important. More frequent dosage of oxazepam is needed to maintain adequate serum concentrations.

It is unclear why some benzodiazepines are promoted as antianxiety agents while others are used almost exclusively as hypnotics. Since a large enough dose of any benzodiazepine will produce sleep, it is doubtful that intrinsic neuropharmacologic differences account for the anxiolytic-hypnotic distinction. Pharmacokinetic differences, however, may well explain the distinction. Long-acting drugs with active metabolites are unsuitable as night-time hypnotics, since central nervous system depression can persist well into the next day. Rapidly metabolized derivatives, on the other hand, can be given in adequate hypnotic doses with minimal residual sedation.

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Genetics and Psychoactive Drugs

Werner Kalow, M.D.

Genetics deals with variation. Variation of drug effects can arise in two different ways. There may be differences in how the body disperses the drug and how the drug affects the body. The first of these two alternatives may be described under the heading "drug metabolism and disposition", and will be reviewed first.

DRUG METABOLISM AND DISPOSITION

Drugs Metabolized by the Mixed Function Oxidase in Liver.

These enzymes (often referred to as microsomal enzymes) are responsible for the biotransformation of the great majority of drugs. Table I shows a number of drugs which undergo metabolic conversion by these enzymes, and the ranges of their plasma half-lives observed during the study of different subjects.[1,2] The half-lives vary over a two- to ten-fold range for different drugs, even among the small numbers of test subjects. By contrast, as Wagner[3] has recently shown, repeated investigations of the same drug in the same subject yield half-lives which rarely differ by more than a factor of 2, and it remains obscure how much of these differences can be accounted for by the experimental error inherent in each single half-life determination. Wagner's review covers five drugs (not counting ethanol), 36 subjects, and between 2 and 6 equivalent determinations of the plasma half-life of a given drug per person. The study permitted 217 comparisons between two half-life determinations of the same drug within one person. Differences which exceeded a factor of two occurred only 7 times among the 217 opportunities. In most of the studies, between 3 and 6

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subjects participated, that is too few to permit a proper assessment of inter-subject variation. Only in a study with clindamycin were as many as 12 subjects used, and the half-life was determined 3 times in each. The inter-subject variation exceeded the intra-subject variation by at least 5 times. (Experimental error and intra-subject variation were not separated.)

TABLE I

VARIABILITY OF PLASMA HALF LIFE OF DRUGS*

Drug	Observed range (hours)	Number of persons tested	Ref.
Amobarbital	14-42	10	9
Glutethimide	8-18	10	9
Methaqualone	0.7-4	7	10
Diazepam	9-53	22	11
Phenylbutazone	29-175	28	12
Sulfinpyrazone	3-6	10	9
Antipyrine	5-35	33	13
Dicoumarol	7-74	28	14

*See refs 1, 2

Much of this person-to-person variation of drug elimination is inborn and of genetic origin. For instance, it has been shown, with the help of twin studies, that the plasma half-lives of phenylbutazone,[4] dicoumarol,[5] antipyrine[6] and amobarbital[7] are basically under genetic control, and the same conclusion was reached by a study of steady-state levels of nortriptyline (Fig. 1).[8] This does not exclude secondary modification by enzyme inducers or inhibitors. There are additional family studies for phenylbutazone,[9] dicoumarol[10] and nortriptyline,[11] which indicate that the control is multigenic. That is, the control depends on numerous genes, and there is no simple Mendelian pattern of inheritance within most families. However, genetic control implies that the basic ability of any given subject to deal with a particular drug tends to be constant in the absence of secondary modification. There are several studies in the literature which directly or indirectly confirm this postulate.[9, 12-15]

To what extent the control is exerted upon the drug metabolizing enzymes, or whether the access to these enzymes is genetically controlled is a question which cannot be answered with certainty for most of the drugs named above. Genetic defects of drug oxidation can occur and have been demonstrated in exceptional families with acetophenetidin,[16] or diphenylhydantoin.[17] Furthermore, there are arguments in favour of the assumption that the antipyrine half-life directly reflects the enzymatic activity in the liver.[18] If this assumption is correct, and as the twin

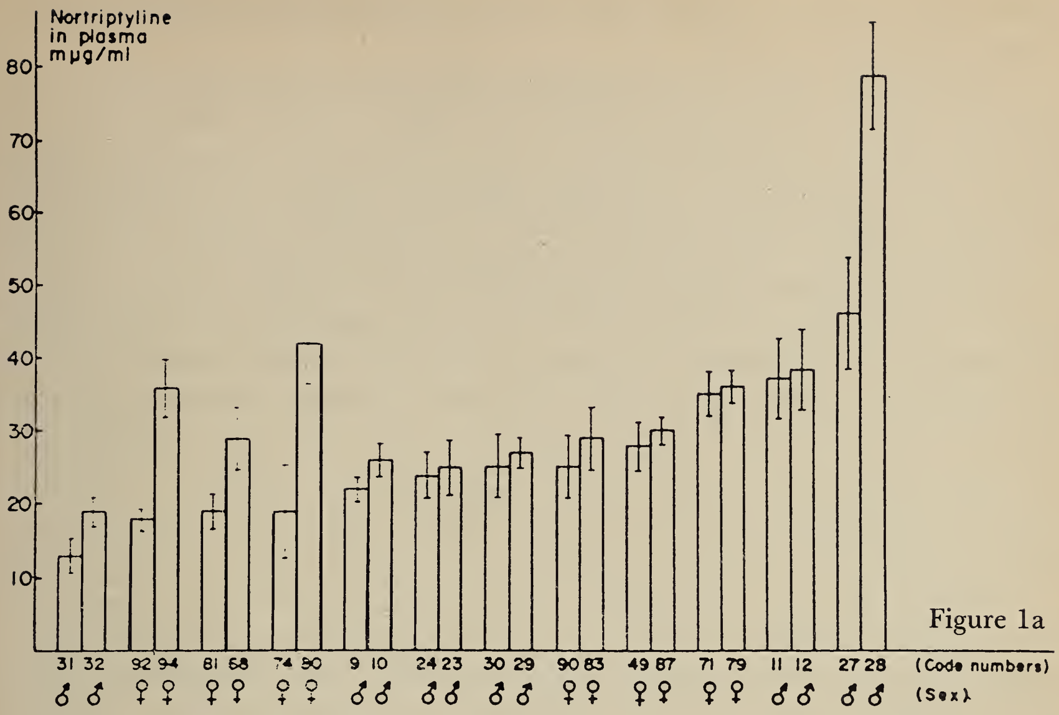

Figure 1a

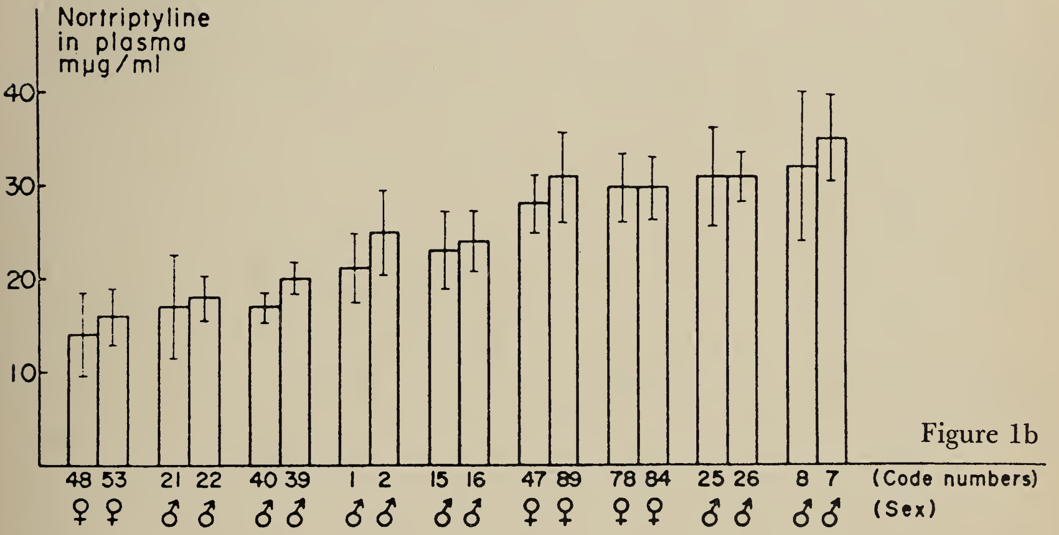

Figure 1b

Figure 1: *Steady-state plasma levels of nortriptyline in fraternal (1a) and identical (1b) twins.*

The plasma levels were measured repeatedly following a standardized schedule of regular intake of nortriptyline. The subjects were free of other drugs. The greater similarity within pairs of identical than within pairs of fraternal twins indicates that the plasma levels are controlled much more by genetic than by environmental factors. Auxiliary studies permitted the conclusion that the activity of the drug metabolizing enzymes in liver, the volume of drug distribution within each person, and the binding of the drug to plasma proteins were all independently subject to genetic control.

From: Alexanderson et al. Br Med J[8]

studies with antipyrine[16] indicate, then the activity of the mixed function oxidases is under close genetic control.

Thorough investigation of nortriptyline[1] in Sweden showed clearly three levels of genetic control. Some genetic control of plasma protein binding could be established. There was genetic control of the distribution volume of the drug. Finally — as far as is possible to conclude from *in vivo* studies — the activity of the metabolizing enzymes was genetically controlled. The number of genes exerting this control in man did not seem to be large, perhaps no more than 2 or 3.

If one accepts the verdict that the person-to-person variation of the enzyme activity is genetically controlled and accounts for a large part of the individual differences in drug elimination, a new puzzle arises. An extensive recent study[19] of fresh liver biopsy samples showed very little person-to-person variation in the concentration of cytochrome P450 or its reductase. These components of the mixed function oxidases were not variable enough to account for the huge differences in drug disposition between normal subjects. This leaves the possibility of a genetic control of the availability of the co-factor NADPH although this co-factor availability is usually believed not to be limiting except under conditions of starvation.[20]

Whatever the exact mechanisms, the available evidence strongly suggests an extensive genetic control of the rate of microsomal oxidation of drugs. One consequence is that one has to expect more or less genetic control for all drugs which are metabolized by these enzymes. Furthermore, within related groups of drugs, a slow metabolizer of one drug may be expected to be a slow metabolizer of the other. This has been shown to be the case, for the pair nortriptyline and desmethylimipramine,[11] for the pair glutethimide and amobarbital (Fig. 2),[15] and for a number of other pairs of drugs.[1, 13, 15]

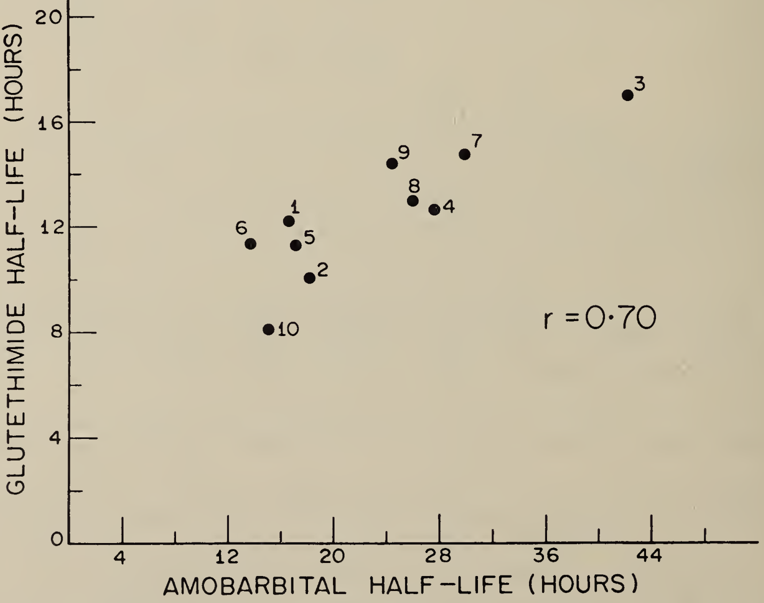

Figure 2: *Correlation between plasma half-lives of amobarbital and glutethimide in 10 male volunteers.*

The determination of plasma levels began 8-10 hours after ingestion of each drug, i.e. after termination of the absorption and early distribution phase. The dose of amobarbital was 130 mg, of glutethimide 500 mg. The interval between the two drug intakes was at least 2 weeks. (cf.[15]*).*

Extramicrosomal Drug Metabolism

In this category is the cholinesterase deficiency which gives rise to prolonged effects of succinylcholine.[22] Well known is the acetylation polymorphism which was first discovered through studies of the fate of the antituberculous drug, isoniazid. The elimination of this and some other drugs depends on the abundance or deficiency of an acetyltransferase in human liver.[23] Thus, the acetylation of the monoamine oxidase inhibitor, phenelzine, depends on this genetically variable enzyme. In a recent study, the therapeutic action of phenelzine was found in rapid acetylators to be not greater than that of a placebo.[24] On the other hand, it was only in slow acetylators that serious side effects of the drug, like liver damage, were prominent.[25] Since phenelzine can block the metabolism of other drugs,[26] such as meperidine, it is reasonable to assume that interaction between these drugs would occur predominantly in slow acetylators of phenelzine.

It has been claimed that the rate of ethanol metabolism in healthy subjects is predominantly under genetic control. Vesell *et al*[27] measured the disappearance of blood alcohol in 14 pairs of twins, half of them identical, the other, fraternal. Some examples of the data are shown in Figure 3. There is an obvious similarity of the slopes within pairs of identical twins and a dissimilarity within those of fraternal twins. A calculation based on these slopes in the 14 pairs of twins indicated an almost complete heritability of ethanol elimination.[27] However, converting the data in this figure to rates of ethanol metabolism according to Widmark's formula, one obtains the values shown in Table II. The rate of ethanol metabolism is more uniform among all subjects than the raw data indicate. There are still slightly larger differences between fraternal than identical twins, so that genetic control of ethanol metabolism cannot be excluded. Much more striking, however, is the large variation in distribution volumes, so that most of the genetic control seems to be exerted upon the volume of distribution of ethanol in the body. Puzzling remains the fact that in numerous cases the calculated distribution volume exceeds the body volume. This is such an unusual observation with ethanol that the conclusion of a genetic control of ethanol metabolism is not convincing without further investigation.

A Canadian study[28]showed differences of ethanol metabolism between Indians and Eskimos on the one hand, and Whites on the other. Since these differences could not be accounted for by previous alcohol consumption or dietary habits, they were thought to have a genetic basis. Unfortunately this study contains an inconsistency. While the data indicate a higher rate of alcohol metabolism in Whites than in the native populations, it is also stated that prolonged infusion of the same amount of alcohol produced identical blood levels of ethanol in natives and Whites. How could this be if the Whites eliminate ethanol at a faster rate? Thus, this study will require confirmation before the conclusion can be accepted that ethanol metabolism is genetically controlled in normal subjects. However, there is another aspect of genetics and alcohol metabolism which cannot be challenged.

At least 10% of Caucasian subjects have in their liver an atypical alcohol dehydrogenase.[29, 30, 31] This enzyme is distinguished from the normal variety by its lower pH optimum, and by the fact that it is capable of oxidizing ethanol at a much higher rate than does the normal enzyme.[29] *In vitro,* the atypical dehydrogenase acts upon ethanol seven to eight times as fast as does the normal enzyme. Figure 4 shows that this *in vitro* difference is not reflected *in vivo.*[29] The curves indicate blood

TABLE II

ETHANOL METABOLISM IN TWINS

	Body weight (kg)	Rate of decrease of blood levels (g/1/hr)	Initial blood levels (Extrapolated) (g/1)	Ethanol* consumed (g)	Volume of distribution (1)	Ethanol metabolism (g/kg/hr)	Volume of distribution in % body weight (%)
Fraternal							
FD	76	0.24	1.15	56.32	48.97	0.155	64.43
PD	76	0.11	0.48	56.32	117.33	0.170	154.38
SA	45	0.11	0.72	33.35	46.32	0.113	102.93
FM	80	0.16	0.68	59.28	87.18	0.174	108.98
HH	43	0.21	1.28	31.86	24.89	0.122	57.88
PM	43	0.14	0.73	31.86	43.64	0.142	101.49
Identical							
B	62	0.20	0.97	45.94	47.36	0.153	76.39
S	50	0.20	0.95	37.05	39.00	0.156	78.00
GeL	62	0.13	0.65	45.94	70.68	0.148	114.00
GuL	59	0.15	0.70	43.72	62.46	0.159	105.86
B.V.	77	0.15	0.84	57.06	67.93	0.132	88.22
J.V.	67	0.15	0.78	49.65	63.65	0.143	95.00

* 1 ml 95% Ethanol/kg. Spec. gravity = 0.78. Kg x 0.95 x 0.78 = g.
(Data calculated from Figure 1 and Table I of Vesell *et al.*[27]

alcohol of 3 subjects after a test drink. Curve 1 represents the subject with normal, curve 2 with atypical alcohol dehydrogenase. The alcohol disappearance rates are very similar.[29, 31] By contrast, ethanol disappears unusually fast in subject 3, a patient with glycogen storage disease. The relatively high pyruvate level in this patient and the consequent abundance of the co-factor NAD, can account for the high rate of ethanol metabolism. In short, the supply of co-factor determines the rate of ethanol metabolism.

Since the co-factor NAD is used up during ethanol oxidation (Fig. 5), the levels of lactic acid in liver and blood tend to rise.[32] This may lead to an attack of gout in genetically predisposed subjects,[33] or the resulting acidosis may produce sickling in subjects with sickle cell disease.[34] The rise of lactic acid may be greater than normal in diabetics, particularly in diabetics who receive phenformin for the reduction of blood sugar.[35] On the other hand, in an adult with inherited chronic lactic acidosis, the intake of ethanol produced coma, a cardiac attack, and prolonged muscular weakness.[36]

The presence of atypical alcohol dehydrogenase may be of clinical importance even if it does not enhance the normal rates of alcohol metabolism. Ugarte and Valenzuela[37] investigated the type of alcohol dehydrogenase in 80 alcoholics and found an unusually high occurrence of the atypical enzyme in subjects with fatty liver. This observation cannot be explained at the present time but it certainly deserves further investigation.

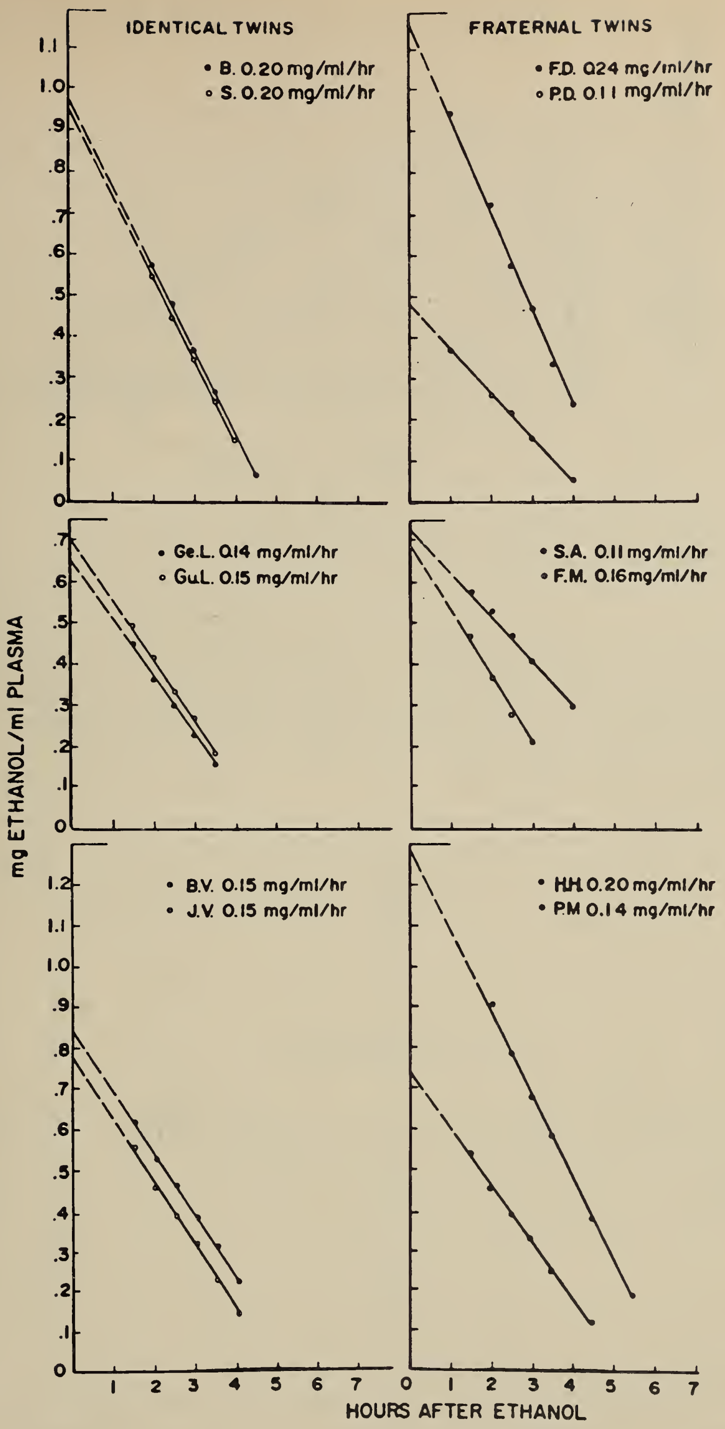

Figure 3: *Ethanol disappearance from the plasma of twins.*

Close similarity of identical twins in rates of ethanol removal and large intrapair differences between fraternal twins. Each subject received orally 1 ml of 95% ethanol per kg of body weight. The figure presents examples from a larger set of data. (From Vesell et al[27]).

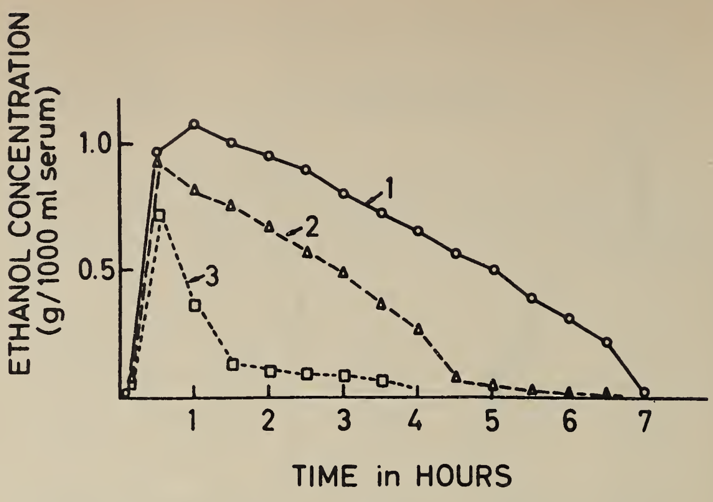

Figure 4: *Blood levels of alcohol in three subjects after a test drink.*

Curve 1 represents a subject with normal, curve 2 with atypical alcohol dehydrogenase. The enzyme of the 2nd subject oxidizes alcohol in vitro about 7 times as rapidly as that in the first subject but this difference is not reflected by the disappearance of alcohol in vivo. Curve 3 shows the rapid alcohol disappearance in a case of type I glycogen storage disease. The data suggest that alcohol metabolism is governed by the co-factor supply and not by the alcohol dehydrogenase.

From: von Warburg and Schürch.[29]

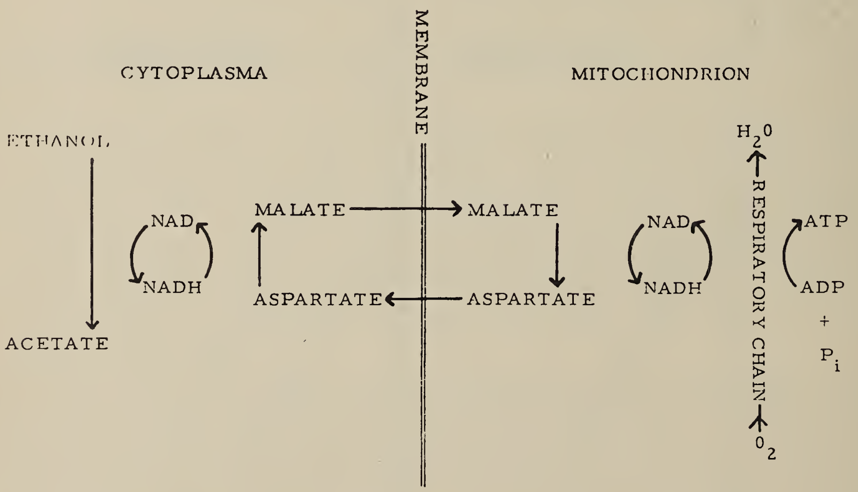

Figure 5: *Biochemical elements in alcohol metabolism.*

Oxidation of ethanol to acetaldehyde by alcohol dehydrogenase, as well as the further oxidation of acetaldehyde to acetate, require the co-factor NAD (nicotinamide adenine dinucleotide) which is thereby reduced to NADH. Re-oxidation of the latter depends on mitochondrial respiration. Since neither NAD nor NADH can cross the mitochondrial membrane, several cross-membranal shuttle systems are in operation of which only the malate-aspartate system is indicated.

The rate of ethanol metabolism depends on the supply of NAD. The scheme shows factors which govern this supply and which thereby might govern alcohol oxidation.

Genetic Variation of Drug Actions Which Are Not, or Probably Not, Dependent on Metabolism

Good examples of this kind[38] of effect are the hemolysis in cases of glucose-6-phosphate dehydrogenase deficiency, the genetic resistance to the anticoagulant effect of Warfarin, or the production of malignant hyperthermia by general anaesthetics. No equivalent information is available in regard to psychoactive drugs. However, there is an interesting example concerning alcohol.

Wolff demonstrated that ethanol produced flushing and other signs of vasodilatation much more readily in Oriental[39, 40] and American Indian[40] subjects than in Caucasians. These observations were recently confirmed and extended by Ewing *et al.*[41] The vasodilatation was not only visible but could also be measured by density recording from the earlobe, and was reflected in alterations of heart rate. Since these racial differences in vasodilatation was present not only in adults but also in newborns and young infants,[39] they are obviously inborn and likely genetic. Since the vasodilatation in susceptible subjects occurred within a few seconds after intravenous alcohol injection,[41] the reaction seems to be due to the ethanol itself and not due to acetaldehyde accumulation.

Orientals and Whites differed also in their subjective perception of alcohol effects. A significantly larger proportion of Orientals than Caucasians reported a feeling of discomfort, muscle weakness, dizziness, and (only in Wolff's study) sleepiness or actual falling asleep. The Caucasians were more likely to report being relaxed, happy, confident, or alert.

Ewing *et al*[41] concluded that the unpleasant reactions account to a large extent for the rarity of alcoholism among Orientals. However, the same reactions do not deter American Indians from consuming alcohol.

DISCUSSION

I did not describe the evidence for genetic components in the predispositions to become an alcoholic,[42, 43] a smoker of tobacco, or an excessive user of coffee.[42] In all these cases, one cannot yet define the nature of the genetic component, and the environmental component is the one which offers opportunities for medical, social, or legal manipulations more than does the genetic one.

Most appropriate for this paper would have been precise definition of genetic differences of the central nervous system in man which would cause differences in the response to psychotropic drugs. To my knowledge such data do not exist. This is probably due to a lack of investigation or means of investigation rather than due to the non-existence of such cases. Professor Murray Jarvik told me many years ago about his studies with LSD, long before it became a drug of abuse. Many subjects had hallucinations with as little as 20 μg ; subjects who did not hallucinate on exposure to this small dose did not do so even if he increased the dose to 200μg; he could not increase the dose any further because he then started to see cardiovascular effects. A thorough investigation into the cause of these differences of response might have produced most valuable genetic data but one cannot be surprised that this investigation was never conducted.

In my paper, I emphasized genetic differences in drug elimination since these are known to be able to produce major differences in drug response. I outlined genetic concepts concerning drugs which are metabolized to an appreciable extent by the mixed function oxidases in the liver; the investigations are only in a pioneering stage but the uncovered principles may be valid for most drugs including psychotropic agents. I found myself forced to caution against the claim of proof of a major genetic control of alcohol metabolism in healthy human subjects; on the other hand I could name several genetic diseases which may be affected through alcohol metabolism. Leaving the subject of drug metabolism, I could at least indicate that several side-effects of alcohol fulfil conditions of a genetically controlled trait. It remains to be seen to what extent the main and crucial effects of alcohol are genetically controlled.

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Modification of Drug Effects After Chronic Ethanol Consumption

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ABSTRACT A brief review of the literature pertaining to the influence of chronic alcohol consumption on drug effects is presented. It is apparent that chronic ethanol consumption decreases the sensitivity of the brain to central nervous system depressants. Furthermore, studies on man and animals indicate that chronic ethanol treatment influences drug distribution within the body and increases the rate of elimination of drugs from the body. This latter effect has been attributed to an increased capacity of the liver to metabolize drugs. In this respect the increased elimination of tolbutamide, warfarin, diphenylhydantoin, meprobamate and pentobarbital in alcoholic subjects has been noted. It is striking, however, that so few drugs have been investigated for alterations in effect when given to chronic alcoholics. It is strongly recommended that drugs, such as the tricyclic antidepressants, be studied for altered pharmacokinetics in individuals with chronic alcohol abuse.

The purpose of this paper is to review briefly the literature relating to the influence of chronic ethanol consumption on the distribution and elimination of drugs. Considerable effort has been directed into investigating the interactions of ethanol with a variety of central nervous system active drugs. These have, however, dealt mainly with the acute effects of the drugs plus ethanol. Little attention has been directed towards understanding the effects of chronic ethanol consumption on drug responses. Evidence exists to suggest that chronic alcohol ingestion may alter drug effects. For example, alcoholics are more difficult to anesthetize than control subjects. Literature describing this effect dates back many years. In 1940 Ahlquist and Dille[1] administered ethanol intraperitoneally to rabbits daily for 15 to 25 days. The dose of ethanol was 1.5 gms per kilogram of body weight per day. Forty-eight hours after stopping ethanol administration the rabbits were administered sodium pentobarbital, sodium hexobarbital or ether. The ethanol-pretreated rabbits were more tolerant to the anesthetics than were control rabbits not given ethanol. This paper represents one of the earliest controlled studies demonstrating that tolerance

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to central nervous system depressants accompanies chronic ethanol consumption. The extent to which chronic ethanol ingestion interferes with the actions of other drugs is not clear. This paper will discuss the effects of long-term ethanol administration on drug absorption, distribution, action on receptors and elimination. A few relevant papers will be cited. No attempt will be made to mention all the articles published in the field.

Absorption

Few, if any, studies have explored the influence of chronic ethanol consumption on the absorption of drugs from the gastrointestinal tract. A preliminary study[2] into the effects of the acute use of ethanol on the absorption of promethazine from the stomach and small intestine of rats concluded that it was without effect, although it increased the absorption of phenobarbital and pentobarbital from the stomach of the rat. These results do not, however, indicate whether chronic ethanol ingestion influences drug absorption. This is a fertile area for research.

Distribution

Several papers have been published that suggest the chronic administration of ethanol influences drug distribution. Frahm *et al.*[3] supplied ethanol solutions of 5%, 10%, or 15% to guinea pigs as the only source of liquid. Every 7 days the animals were challenged with either pentobarbital, methitural or hexobarbital. The duration of narcosis produced by each drug was reduced in the ethanol-treated animals and fell in proportion to the concentration of the ethanol administered. One result, taken from this paper, is given in Table I. From these results it can be seen that the administration of a 5% solution of ethanol daily for one week reduced the sleeping time produced by pentobarbital from 110 to 55 minutes. One explanation for these results was advanced by Seidel *et al.*[4] who suggested that the administration of a 5% alcohol solution to guinea pigs in place of drinking water for one week reduced the diffusion of pentobarbital from the blood into the brain. These workers administered a 5% solution of ethanol to guinea pigs for one week. At the end of this time the animals were administered pentobarbital i.p. Thirty minutes after the injection the blood levels of the barbiturate were significantly higher in the alcohol-treated guinea pigs (Table II). Fifty-five and 110 minutes after administration there was no difference in the blood concentrations of pentobarbital between the two groups. Support for the contention that chronic ethanol consumption may diminish the diffusion of pentobarbital from the blood into the brain is obtained from the brain levels of the barbiturate 55 minutes after treatment (Table III). A higher concentration of pentobarbital was found in the brains of the control animals.

TABLE I

INFLUENCE OF ADMINISTERING A DRINKING SOLUTION CONTAINING 5% ETHANOL FOR ONE WEEK ON THE SLEEPING TIME OF GUINEA PIGS GIVEN 20 mg/kg OF PENTOBARBITAL, I.P.

	Control Guinea Pigs	Chronic Alcohol-Treated Guinea Pigs
Sleeping Time	110 min.	55 min.

Results taken from Frahm M, et al: Arzn Forsch 12: 1055-1056, 1962.

TABLE II

INFLUENCE OF ADMINISTERING A DRINKING SOLUTION CONTAINING 5% ETHANOL TO GUINEA PIGS FOR ONE WEEK ON THE PENTOBARBITAL CONCENTRATIONS IN SERUM (μg/ml) FOLLOWING A DOSE OF 20 mg/kg OF PENTOBARBITAL I.P.

	Control Guinea Pigs	Chronic Alcohol-Treated Guinea Pigs
30 minutes	15.4 ± 5.6	22.1 ± 7.0 ($P<0.02$)
55 minutes	14.1 ± 2.9	15.5 ± 4.4
110 minutes	13.3 ± 2.5	15.2 ± 4.8

Results taken from Seidel G, et al: N S Arch exp Path Pharmakol 247: 312, 1964.

TABLE III

INFLUENCE OF ADMINISTERING A DRINKING SOLUTION CONTAINING 5% ETHANOL TO GUINEA PIGS FOR ONE WEEK ON THE PENTOBARBITAL CONCENTRATION IN BRAIN (μg/g) FOLLOWING A DOSE OF 20 mg/kg OF PENTOBARBITAL, I.P.

	Control Guinea Pigs	Chronic Alcohol-Treated Guinea Pigs
55 minutes	15.3 ± 3.3	10.8 ± 4.3

Results taken from Seidel G, et al: N S Arch exp Path Pharmakol 247: 312, 1964.

The results of Seidel *et al.*[4] were criticized in a paper by Kalant, Khanna and Marshman[5] who administered ethanol, as 5.1% of the diet, to male Wistar rats for 2 weeks. The daily intake of ethanol in this study was 10-12 gms per kilogram of body weight. Control rats received an equivalent liquid diet in which the ethanol was replaced by an equicaloric amount of sucrose. After two weeks both liquid diets were replaced by tap water for 24 hours. Pentobarbital was then injected intraperitoneally in a dose of 30 mgs per kilogram. The results are presented in Tables IV and V. Chronic ethanol treatment decreased the sensitivity of rats to pentobarbital, with the onset of sleep requiring longer time and the duration of sleep being shorter. The delayed onset of sleep in the ethanol-treated rats correlated well with the lower levels of pentobarbital in the plasma and brain of these animals 15 minutes after injection. No evidence was found in this study to support the contention that chronic ethanol treatment diminishes the diffusion of pentobarbital from the blood into the brain. The blood/brain concentration ratio was almost identical in the two groups, averaging 1.41 ± 0.19 in the ethanol-treated animals and 1.42 ± 0.07 in the controls. At 30 minutes or later, there was no longer any difference between groups, with respect to either brain or plasma concentrations of pentobarbital. In view of the observation that the brain concentrations of pentobarbital were not significantly different in the two groups of rats from the 30 to 60 minutes, it is likely that the decreased sleeping time of the ethanol-treated group reflected a decreased tissue sensitivity.

TABLE IV

EFFECT OF CHRONIC ETHANOL TREATMENT ON ONSET AND DURATION OF SLEEP INDUCED BY PENTOBARBITAL (30 mg/kg i.p.) IN RATS

	N[a]	Body Weight	Onset of Sleep	Duration of Sleep
		g	min	min
Chronic ethanol group (10-12 g/kg daily for 14 days)	17	290 ± 7[b]	6.05 ± 0.38	28.6 ± 2.54
Pair-fed controls	17	295 ± 7	4.76 ± 0.20	48.9 ± 2.61
P[c]		N.S.	$<.005$	$<.001$

[a] N indicates number of animals.

[b] Values shown are mean ± S.E.M.

[c] P refers to the significance of differences between chronic ethanol group and the pair-fed controls as estimated by t test for paired data. N.S., not significant ($P > .05$).

Results taken from Kalant H, et al: J Pharmacol Exp Ther 175: 318-324, 1970.

TABLE V

PENTOBARBITAL LEVELS IN BLOOD AND BRAIN OF ETHANOL-TREATED RATS AND PAIR-FED CONTROLS AFTER I.P. INJECTION OF PENTOBARBITAL, 30 mg/kg

Time After Injection	Pentobarbital Level in: Chronic Ethanol Group		Pair-Fed Controls	
	Plasma µg/ml	Brain µg/g	Plasma µg/ml	Brain µg/g
min				
15	9.76 ± 0.92 (5)[a]	13.34 ± 1.46 (5)	11.98 ± 0.50 (5)	16.92 ± 0.46 (5)
30	8.85 ± 0.67 (6)	12.08 ± 1.03 (6)	8.99 ± 0.89 (6)	12.24 ± 0.75 (6)
45	5.72[b] ± 1.10 (5)	8.25 ± 1.50 (6)	8.60 ± 0.54 (5)	10.47 ± 0.65 (6)
60	5.41 ± 1.04 (5)	7.11 ± 1.61 (6)	6.00 ± 0.77 (5)	8.17 ± 0.70 (6)

[a] Values shown are mean ± S.E.M., with number of animals per group in parentheses. Brain and plasma pentobarbital concentrations in individual ethanol-treated rats and their pair-fed controls were compared by t test for paired data. The differences in pentobarbital concentrations in plasma and brain at 15 minutes were significant ($P < .05$ in both cases); no significant differences were found at any of the other times tested.

[b] This mean includes one unusually low value of 2.21 g/ml.

Results taken from Kalant H, et al: J Pharmacol Exp Ther 175: 318-324, 1970.

The delayed onset of sleep,[5] together with the lower plasma and brain levels of pentobarbital 15 minutes after i.p. injection, may reflect either a reduced rate of absorption of the barbiturate from the peritoneal cavity or an altered distribution of the drug in the body during the initial moments after treatment. Recent studies from our laboratory by Patel and Johnson[6] support this latter possibility. Rats were administered ethanol as 5.1% of their diet for 2 weeks in the same manner as the studies of Kalant *et al.*[5] Control rats were fed an equicaloric amount of sucrose. On Day 15, 24 hours prior to challenge with thiopental, the respective diets were removed and the animals were given water. Thiopental, 25 mg/kg, was administered i.v. into the tail vein. Anesthesia was almost immediate. The control group slept for 11.9 minutes ± 1.2 (S.E.M.) and the alcohol-treated group slept for 8.3 minutes ± 0.9 (S.E.M.) ($P<0.01$). Blood samples were taken by cardiac puncture at 1, 5 and 10 minutes after injection. The control group showed significantly higher blood levels of thiopental 1 minute after injection than did the alcohol-treated group (Table VI). At 5 and 10 minutes no difference between the two groups was seen. From these results it may be inferred that chronic ethanol ingestion alters the initial distribution of thiopental with the result that lower blood levels of the drug are seen. This phenomenon could account, at least in part, for the delay in sleep seen in animals given a barbiturate after a period of alcohol treatment.

The results of Patel and Johnson[6] also support the concept that chronic ethanol ingestion diminishes the sensitivity of the brain to barbiturates. The ethanol-treated rats awoke at 8.3 minutes, when the blood levels of thiopental were in excess of 19.6 μg/ml (Table VI). The controls awoke at 11.9 minutes, when the concentration of thiopental was below 17.8 μg/ml.

TABLE VI

THIOPENTAL LEVELS IN BLOOD OF ETHANOL-TREATED RATS AND PAIR-FED CONTROL AFTER I.V. INJECTION OF THIOPENTAL 25 mg/kg.

	1 minute	5 minutes	10 minutes
Control Group: (7)			
Mean ± S.E.M.	33.3±1.3	20.8±2.2	17.8±2.4
Alcohol Treated Group: (7)			
Mean ± S.E.M.	27.7±1.3	22.9±1.3	19.6±1.9
Level of Significance Between Means of two Groups			
	$p < 0.01$	N.S.	N.S.

[a] Rats were administered ethanol as 5.1% of their diet for 2 weeks. Control rats received an equicaloric amount of sucrose. Values are expressed as μg of thiopental per ml of blood.

Elimination

Evidence has been accumulating over the past few years to implicate chronic ethanol consumption as a causative factor in the increased rate of elimination of drugs in both man and animals. Kater *et al*, in 1969[7] described an increased rate of tolbutamide metabolism in alcoholic patients. Two groups of subjects were selected by the investigators. The first group consisted of 20 alcoholic patients who were admitted to hospital during a heavy drinking episode. They included 18 males and 2 females. The age of this group ranged from 18 to 58 years and their mean weight was 63.0 kilograms. Each subject had consumed at least 250 gms of ethanol daily for two years or longer. Despite this continued heavy intake of alcohol, there was no clinical evidence of hepatic damage except for hepatomegaly in some patients. Results of liver function tests were normal in all patients at the time of the study. There was no evidence of cirrhosis or liver failure in any patient. Liver biopsies were performed on 7 patients. Results of 3 biopsies were normal and the results of 4 showed mild fatty infiltration. The control group consisted of 10 nonalcoholic subjects. They included 6 men and 4 women and ranged from 24 to 60 years with a mean weight of 62.2 kilograms. They were either healthy hospital attendants, sick subjects, or patients who were admitted to the hospital with unrelated disease. Sufficient time was allowed after admission of the patient to the hospital for the alcohol to be cleared from the body. On the day of the study each subject was given breakfast. The test dose of 1 gm of tolbutamide was injected intravenously over a two minute period. Thirty minutes were allowed for equilibration and distribution of the drug. Plasma samples were obtained after 30 and 60 minutes, then hourly for 6 hours after the injection. The individual results of the biological half life of tolbutamide for the alcoholic and control groups are shown in Table VII. Tolbutamide in the alcoholic had a mean half life of 165.4 minutes as compared with the mean half life of 351 minutes in the nonalcoholic control. The difference was highly significant. Subsequent work by Kater and others[8] indicated that the half lives of both warfarin and diphenylhydantoin were significantly reduced in alcoholic patients during the time that they were actually free of alcohol. These results are also presented in Table VII. The conclusion of these workers was that the heavy use of alcohol stimulates the metabolism of a variety of microsomally metabolized drugs to a clinically important degree.

TABLE VII

MEAN HALF LIVES (±S.D.) OF TOLBUTAMIDE, WARFARIN AND DIPHENYLHYDANTOIN IN SUBJECTS WITH A HISTORY OF CHRONIC ETHANOL CONSUMPTION AND IN CONTROL SUBJECTS

	Controls	Alcoholics	P
Tolbutamide	350.6±130.6 (10) Minutes	165.4±33.5 (20) Minutes	< 0.0005
Warfarin	41.0± 19.2 (10) Hours	26.5±13.3 (15) Hours	< 0.02
Diphenylhydantoin	23.5± 11.0 (76) Hours	16.3± 6.8 (15) Hours	< 0.02

Results taken from Kater RMH, et al: J Amer Med Assoc 207: 363-365, 1969; and Kater RMH, et al: Gastroenterology 56: 412, 1969.

This conclusion is supported by the work of Misra *et al*,[9] who administered 7 pairs of rats isocaloric diets with or without ethanol. Five to 16 hours after the last feeding,^{14}C-meprobamate (250 mgs per kg i.v.) was given and the radioactivity in venous tail blood measured every 30 minutes for 5-7 hours. The half life of the radioactivity in the control rats was 254 minutes and in the ethanol-treated animals 138 minutes (Table VIII). The results of this study must be treated with caution because it is not apparent whether the authors measured the rate of clearance of unchanged meprobamate or merely measured the rate of clearance of radioactivity from the blood. These authors also reported a similar effect in testing the half life of meprobamate in the venous blood of alcoholic and control subjects. These observations, from *in vivo* studies, are supported by the results of *in vitro* experiments. Feeding rats for 1 month with ethanol doubled the capacity of liver slices to convert meprobamate into polar metabolites (Table IX). The increased capacity of the liver to metabolize meprobamate parallels the stimulation, by ethanol, of microsomal aniline, pentobarbital and benzpyrene hydroxylases.

TABLE VIII

EFFECT OF CHRONIC ETHANOL TREATMENT ON THE HALF-LIFE OF RADIOACTIVITY IN RAT BLOOD FOLLOWING THE I.V. INJECTION OF ^{14}C-MEPROBAMATE

Values are expressed in minutes ±S.E.M.

Control Rats	Rats Treated Chronically with Ethanol for 1 Month	P
254 ± 36 (7)	138 ± 19 (7)	< 0.02

Results taken from Misra PS, et al: Gastroenterology 58: 308, 1970.

TABLE IX

EFFECT OF CHRONIC ETHANOL TREATMENT ON THE ABILITY OF RAT LIVER SLICES TO METABOLIZE MEPROBAMATE, VALUES EXPRESSED AS μg OF MEPROBAMATE METABOLIZED PER g OF LIVER PER HOUR ±S.E.M.

Control Rats	Rats Treated Chronically with Ethanol for 1 Month	P
58 ± 8 (8)	99 ± 9 (8)	< 0.001

Results taken from Misra PS, et al: Gastroenterology 58: 308, 1970.

Rubin *et al.*[10] and Rubin and Lieber[11] reported that chronic ethanol administration to rats increased hepatic smooth endoplasmic reticulum, microsomal drug metabolizing enzymes and the microsomal ethanol oxidizing system. In man, ethanol feeding produced hypertrophy of the smooth endoplasmic reticulum and increased hepatic pentobarbital hydroxylase. In a later study by the same group,[12] volunteers were given ethanol (42-48% of the total calories) for 3 to 4 weeks. The plasma half life of several drugs, including pentobarbital, was substantially decreased after ethanol feeding and the rate of blood ethanol clearance was accelerated.

In view of these results it is perhaps appropriate to speculate that chronic alcohol consumption will lead to an increased rate of metabolism of a wide range of drugs. The proof of this must await further research.

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Cardiovascular Responses to Marihuana in Man

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ABSTRACT Forearm arterial and venous pressures, forearm blood flow and heart rate were recorded in 28 supine subjects, matched by sex and cannabis experience, who received 600 mg marihuana placebo and active marihuana under single- and double-blind conditions. Derived functions such as "dp/dt", regional arterial resistance and venous compliance were calculated from these variables. Under the conditions of these experiments, marihuana placebo produced no intoxication or consistent physiological responses. Active marihuana produced intoxication in all cannabis-experienced and half the non-experienced subjects. Cardiovascular responses still occurred in the absence of intoxication, indicating that they are not psychogenically mediated. Inhibition of vagal tone may contribute to the tachycardia noted. There is some suggestion that reflexly mediated sympathetic responses may be lessened by marihuana.

The acute administration of marihuana in man appears to produce few consistent physiological effects except for those on the cardiovascular system. Regardless of the route of administration, a dose-dependent tachycardia has been reported by all investigators; conjunctival injection has been another consistent finding. A fall in arterial blood pressure is usually produced in the upright subject, but when the subject is supine, cannabis derivatives either have no effect or cause a slight increase.[1-3] Such observations suggest dilatation of capacitance vessels with secondary effects on venous return and cardiac output. An increase in limb blood flow with possible interference with peripheral vascular reflex responses in man has been reported recently.[3,4] There is also considerable evidence to suggest an effect of marihuana on both the sympathetic[4-7] and parasympathetic divisions of the autonomic nervous system.[8]

Unfortunately, the differing experimental protocols used in previous studies and the fact that drugs were commonly given orally, make it difficult to assess the dimensions or importance of changes noted. The present study employs an experimental design that overcomes many of the limitations of earlier studies.

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The interim report of the Canadian Commission of Inquiry into the Non-Medical Use of Drugs[9] notes: "Subjects must be similar to the general population. . .Variables such as age, sex and social class. . .must be taken into careful consideration. . .The dose, mode of administration and the general circumstances of the study must be relevant to the pattern of use in the general population". The Commission also discusses the need for the use of a placebo, single blind, and where possible, double blind techniques. In the subsequent Cannabis report[10] the Commission also presents arguments for employing female as well as male subjects in studies on cannabis derivatives. These factors in experimental design have also been discussed by Weil *et al.*[11] and by Klonoff.[12]

In the design of the present study, considerable emphasis was placed on such factors as: subject selection representative of the general population, a socially relevant psychological set, physical setting and delivered dosage, as well as on consideration of possible sex differences and differences related to prior cannabis use.

METHODS

Subjects

This study was done as part of a broader study which concerned itself not only with the cardiovascular effects of marihuana, but also with sensory and perceptual changes. Volunteers were obtained through carefully worded newspaper articles which indicated that adult subjects of both sexes from all sectors of the community were being sought for studies on marihuana. The number of individuals volunteering totalled 1600. The average age was 30 with a range of 21 to 85; average education was 12.7 years with a range of "no education" to three years postdoctoral training. Males comprised 51% of the applicant pool and females 49%. Virtually all occupations were represented and no one group had disproportionately large representation; students, for example comprised only 20% of the volunteers.

Over the course of 1½ years some 400 of these potential subjects were chosen randomly for interview. At the interview, details of the experimentation were described to the volunteers and after assurance of confidentiality, volunteers were asked about personal usage of non-medical drugs. About 50% of the volunteer population had previous experience with cannabis.

If the subjects were needed for the physiological experiments, volunteers were told at the time of the interview that they could volunteer either for psychological experimentation or for physiological experimentation. It was made quite clear to them that there would only be one exposure to marihuana and while they could volunteer for either "psychological" or "physiological" experiments, they could participate in only one or the other.

All prospective subjects next completed a detailed medical history which included an extensive drug history. A physician then performed a detailed physical examination. Subjects were interviewed by a psychiatrist, who sought evidence of past or present psychopathology.

In addition to exclusion on the basis of the medical and psychiatric evaluations, volunteers were excluded on the following grounds:

1. If they were dependent upon narcotics or were more than occasional users of amphetamines. Volunteers with documented histories of multiple drug use were not excluded unless they were considered psychiatrically unwell or socially unreliable.

2. If they were considered sufficiently unreliable that their compliance with respect to obeying proscription of other drugs was in doubt.

3. If they could be classified neither as "experienced" nor as "non-experienced" with respect to cannabis.

If volunteers were interested in participating in the physiological experiments, they were taken to the room where the experiments would be done. The methodology of the experiments was described in detail and no attempt was made to minimize the procedure. Our subjects were self-selecting insofar as each had the option of volunteering or not volunteering for this particular experiment which they knew to involve insertion of percutaneous needles. The motivation for individuals volunteering for these particular experiments was not examined systematically.

Experimental requirements were for 28 subjects between the ages of 21 and 55 years, equally divided between males and females. The age limit of 55 years was imposed since we were reluctant to insert arterial needles into vessels of subjects who had a greater potential for atherosclerosis. The exclusion of those under 21 was based on medical-legal considerations. Single blind studies required 16 subjects, with equal representation from "experienced" and "non-experienced" groups (see later); double blind studies required 12 subjects, all of whom were to be "experienced".

Our "physiological subjects" were therefore selected both by themselves and by us. In spite of this, their general characteristics were very similar to the total volunteer pool (average age 29.7 vs 30.0; average education 13.2 years vs 12.7 years).

Volunteers were considered "non-experienced" with respect to cannabis products if they had never used them in any form; "experienced" volunteers were defined as those who had used cannabis derivatives on one or more occasions in the past two years and had experienced an unequivocal "high". Volunteers who had tried marihuana or hashish but who had experienced no characteristic symptomatic effects were excluded, since we could, with almost equal justification, consider them either as "experienced" or "non-experienced".

The informed consent form used in these experiments described the methodology and possible complications. This form also indicated that subjects would receive "an inert substance and/or marihuana". In addition, the form constituted an agreement that the subject would not take illicit drugs for 7 days prior to experimentation. Other drugs, such as patent medicines, that might influence the central nervous system or cardiovascular system were also prohibited. Subjects were allowed to continue taking coffee, tea and tobacco but were instructed not to take any alcoholic beverages for 24 hours prior to testing. There was no guarantee that these proscriptions would be followed. Accordingly, both to ensure adaptation to the environment, and to be reasonably confident that the subjects were drug-free, experiments were not done for 6-12 hours after their arrival.

All experiments were conducted in one room of an apartment which contained a living room, kitchen and dining area and a bedroom. While it is doubtful whether we achieved our objective of creating an atmosphere which was totally non-institutional, we did create a setting which was informal and non-threatening. The experimental room was equipped with a modified La-Z-Boy chair upon which the subject would be supine in comfort; he or she also listened to music of choice through stereo earphones. Coloured posters were hung on walls; investigators did not wear laboratory coats. The room was not a constant temperature room but the temperature was consistently about 22°C.

Although women of child-bearing age were not excluded, a pregnancy test was done the day of the proposed experimentation. All procedures were carried out by at least two physicians with emergency equipment on hand. While objective intoxication from marihuana had usually disappeared within 2-3 hours, all subjects were detained a minimum of an additional 9 hours. They were never left without an attendant and were driven home at the end of the observation period. All subjects had repeat medical examinations approximately two weeks after participation.

Materials Administered

The "active" and "placebo" marihuana (solvent-extracted marihuana) were obtained from the Canadian Health Protection Branch which in turn had obtained them from the U.S. National Institute of Mental Health. All active marihuana used bore the same batch number (2-PF-126). On receipt, all material was weighed and "manicured" to remove twigs, etc. but to retain seeds.

The marihuana was stated to contain 1.5% Δ^9-THC and the placebo was said to be free of Δ^9-THC. However, local assay was necessary to establish the accuracy of these values and to determine whether there was any change in the Δ^9-THC content with storage. In addition, it was necessary to ascertain the efficiency of the administration system.

The active marihuana, the placebo, the breathing apparatus and the expired air (passed through cigarette filters) were all solvent-extracted and, using gas liquid chromatography, were assayed against Δ^9-THC standard which was stored under nitrogen.

We have reason to doubt the stated concentration of the N.I.M.H. Δ^9-THC standard, which may be in error by 50%. We believe that the active marihuana may contain 1.0% THC rather than the stated 1.5%. Regardless of absolute amounts, under the conditions of our experiments, approximately 60% - 70% of the Δ^9-THC contained in the marihuana was lost in the breathing apparatus or expired air. Moreover, the placebo did not contain more than 0.01% Δ^9-THC. There was little change with time of the Δ^9-THC content of the marihuana under our storage conditions.

An individual not directly associated with the experimentation weighed and coded all substances.

Administration

The possibility of administering the substances by cigarette was rejected since the tightness of packing in cigarette manufacture, the subject's breathing characteristics, and the variable loss of smoke (particularly in a non-cigarette smoker) might modify the response. Accordingly, we sought a system (a) which could be used by smoker and non-smoker, (b) which incinerated a known quantity of substance under standardized conditions, and (c) in which quantification of the amount of smoke received was possible.

Figure 1 shows the administration apparatus. The smoking device consisted of a small forced air electric grill combustion chamber connected by tubing to a 4 litre glass reservoir bottle connected in turn through tubing to an anesthetic valve which the subject grasped in his mouth. The subject was instructed to breath normally and not to try to prolong retention of the smoke in the lungs. The heating element and a forced air system permitted the material to be combusted under constant conditions. The "reservoir" provided sufficient dilution of the smoke with air so that it was tolerable to the non-smoker. The one-way valve provided a system whereby all expired air was vented to the room.

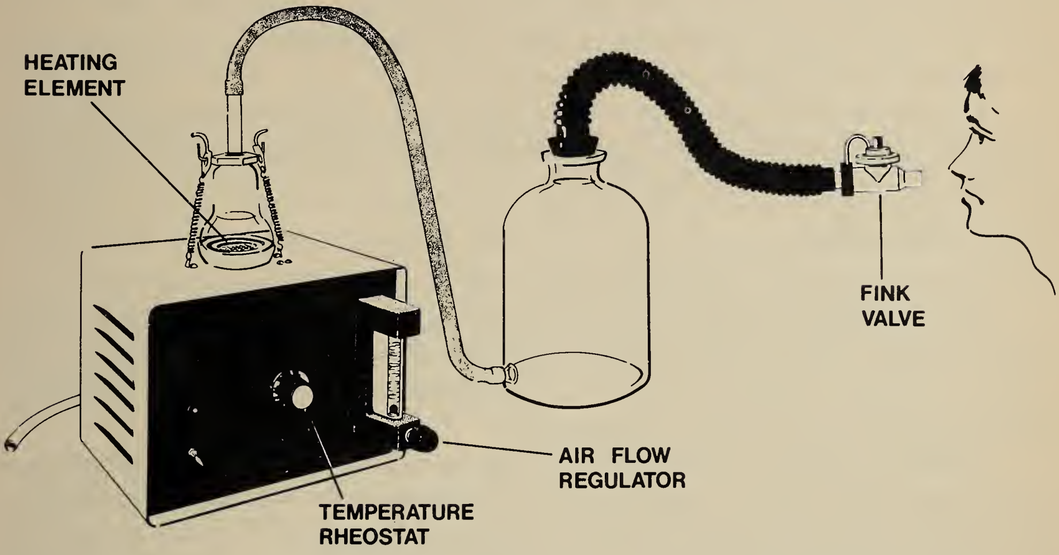

Figure 1. *Details of system used for administration of marihuana and placebo.*

Detailed Protocols

The subject, who was fasted at least three hours prior to the procedure, lay supine in the reclining chair and a 6-inch blood pressure cuff was attached around the upper arm and a 2-inch cuff around the wrist. The upper cuff was attached to a reservoir which inflated the cuff at 40 mm Hg pressure and at a cycle length of 12 seconds (6 seconds "on", 6 seconds "off"). The wrist cuff could be inflated by handbulb (to 240 mm Hg). The pressures achieved in both cuffs were checked by pressure gauges.

A Whitney mercury-in-rubber strain gauge was then attached to the forearm and calibrated. Standard EKG limb leads were attached. In the area of the cubital fossa, a catheter (19 gauge Intercath^{R}) was then inserted retrograde into a vein draining forearm muscle, and advanced until it wedged against a valve. It was then pulled back slightly and taped in place. The arterial catheter (B-D Longdwel^{R} 18 gauge) was inserted antegrade into the brachial artery at the same site. Both venous and arterial punctures were done using minimal amounts of 1% lidocaine (Xylocaine^{R}). The arm was adjusted to heart level and venous and arterial catheters were attached to Statham pressure transducers (P23 BB and P23 Db respectively) calibrated at heart level.

Throughout, the subject was permitted to listen to music of his or her choice through stereo earphones. In addition to occupying the subjects, the music prevented subjects from overhearing and possibly misinterpreting the minimal conversation which took place. The experimenters replied to any comments or queries by the subject but made no attempt to initiate discussion or question the subject once the experiment started. In addition, the room was semi-dark with only sufficient illumination for experimental procedures.

Pilot Studies

The object of pilot experiments was (a) to determine the practicability of making physiological measurements with intervention techniques (e.g. arterial puncture in intoxicated subjects), (b) to determine in a crude way whether body mass, sex, or previous drug experience modified the dose necessary, and (c) to establish whether any changes in protocol would be necessary.

These pilot experiments were done using staff members as subjects. Administration of drugs (varying doses) was "single blind". Each subject recorded his or her comments at the end of the procedure.

Single Blind Studies

The initial preparation of the subject was the same as that described above.

Prior to administration of any material, each subject was told that he or she would receive "half of the material now and the other half after a pause". No subject correctly appraised that he or she would receive placebo then marihuana. The procedure was, therefore, "single blind".

During an initial control period of approximately 15 minutes, basal measurements were made and the following procedures were done sequentially: inflation of wrist cuff to 240 mm Hg (2 minutes), Valsalva manoeuvre (20 seconds), and cold pressor test (opposite hand in water at 1 degree Centigrade for 1 minute). Sufficient time was allowed between procedures for recorded parameters to return to baseline.

The subject breathed through the mouthpiece for at least two minutes before the material was ignited and smoke was delivered. Six hundred mg of marihuana placebo was administered by inhalation over a period of 15 minutes (200 mg every 5 minutes). At the end of this time, the wrist cuff inflation, Valsalva manoeuvre and cold pressor test were repeated and calibration of instruments were checked.

Twenty minutes after cessation of placebo administration, marihuana in a dose of 600 mg was administered over 15 minutes (200 mg every 5 minutes) and the above tests were repeated. The experiment was continued at least 30 minutes after the termination of smoking marihuana. Subjects were told to request termination of the experiment at any point where pain occurred in the catheterized arm. None requested premature termination, although most experienced some discomfort by the end of the procedure.

Double Blind Studies

The protocol for these studies was identical to those of the single blind studies with the exception that (a) only "experienced" individuals were used as subjects, since the single blind studies had shown that in the doses employed, physiological changes were less in "non-experienced" subjects, and (b) the cold pressor test and the Valsalva manoeuvre were omitted.

An investigator not associated with the study coded subjects as follows: (a) marihuana followed by placebo – 4 subjects (2 male, 2 female); (b) placebo followed by placebo – 4 subjects (2 male, 2 female); (c) placebo followed by marihuana – 4 subjects (2 male, 2 female). The code was not broken until the entire series of 12 subjects was done.

In these experiments, following preparation of the subject as described previously, a control period of 15 minutes was allowed, during which time the wrist cuff was inflated (2 minutes). The three tubes of the first substance were then administered at 5 minute intervals. Following this, wrist cuff inflation was repeated and instruments recalibrated. Twenty minutes after completion of smoking the first substance, the three tubes of the second substance were administered, again at 5 minute intervals over 15 minutes. The experiment was terminated 30 minutes after completion of smoking.

Recording of Data

The EKG and strain gauge inputs were led by coaxial cable to Beckman preamplifiers and amplifiers. Outputs were fed simultaneously into a Beckman RM ink-recorder, into a 4-channel oscilloscope, and into a Precision Instrument 6200 FM tape recorder. The ink-writing recorder, run at slow speed, permitted identification of events occurring over many minutes; the oscilloscope permitted observation of rapid events, such as the shape of the pulse wave; finally, recording on tape permitted subsequent time base compression or expansion, as well as computer analysis. All variables were recorded continuously.

Analysis of Data

Following analogue to digital conversion, records were analyzed off-line with a PDP 11 computer which gave the following functions over any preset interval of time: heart rate, mean venous pressure, systolic, diastolic and true mean arterial blood pressures, "dp/dt", calculated forearm blood flows, arterial resistance and venous compliance.

Mean arterial pressure was calculated from the area under the pulse contour curve.

Determination of true dp/dt (*i.e.* the rate of rise of pressure per unit time) would involve pressure recording from the left ventricle. The value of "dp/dt" calculated here was derived from the slope of the brachial artery pulse wave. The value must be considered only an approximation of the true dp/dt.

Forearm blood flows were calculated from the formula:

$$\text{Flow} = 2 \times 60 \times 100 \times \frac{\text{Slope of strain gauge tracing}}{\text{Circumference}} = (\text{ml}/100\text{ ml tissue/min})$$

Arterial resistance was calculated from the formula:

$$\text{AR} = \frac{(\text{mean arterial pressure-venous pressure})}{\text{Flow}} \times 60 \times 1332 = (\text{dynes} - \text{sec/cm}^5)$$

Since cardiac output was not measured, this value is a regional (forearm) resistance, and does not necessarily correlate with total peripheral resistance (TPR).

$$\text{VC} = \frac{\text{Flow}}{\Delta\text{ Venous pressure} \times 1332 \times 60} = (\text{cm}^5/\text{dyne-sec})$$

The paired "t" test was employed in statistical analysis of data.

RESULTS

Data obtained from our previous studies indicated that a fixed dose of 600 mg marihuana would produce intoxication in about 65% of non-experienced subjects and 95% of experienced subjects. (Subjects were considered intoxicated if they exhibited giggling, euphoria, and difficulty with speech.) These studies also indicated that solvent-extracted marihuana was an effective placebo. In pilot experiments done under standardized conditions, the first two subjects, experienced males closely associated with development of the project, received only marihuana in a dose of 600 mg, and both experienced unpleasant intoxication, characterized by anxiety or pain. A similar response was seen in a third subject, a non-experienced female who also exhibited considerable anxiety prior to commencement of the procedure. The next two pilot subjects fortuitously had participated previously in a study which involved similar intervention techniques (but no marihuana administration). Both had previous experience with cannabis derivatives. We reasoned that such individuals would have little initial anxiety and would provide a better indication of the practicability of the procedure. Both of these subjects had no response to placebo but became pleasantly intoxicated with marihuana. Similar responses were noted in a sixth subject, a non-experienced male.

These early experiments convinced us of the importance of "set", and, as a consequence, in subsequent experimentation the additional measures indicated under "Methods" were taken to relieve anxiety. In spite of these measures, we felt that we were still unable to anticipate fully the difficulties which might be encountered. Accordingly, in the interests of subject safety, the first series of experiments was done single blind.

Single Blind Experiments

The characteristics of the subject population are given in Table I. The average age of three of the groups was very similar; the fourth group (non-experienced males) was, on the average, a decade older. This same group was about 11.0 Kg heavier on the average than the experienced males. With the exception of one subject, the weights of experienced and non-experienced females were similar. All groups were roughly similar in educational background; occupations were broadly represented across groups.

All of the subjects used alcohol in moderation (less than 6 bottles of beer/week or less than 6 ounces of spirits). Only two subjects had previous experience with other "street drugs" (amphetamines on one occasion only in each subject; numerous occasions of self administration of hallucinogens – chiefly LSD – in both). Experienced subjects varied considerably in their use of cannabis but all used marihuana or hashish at least once a month and had done so at least three years.

Pilot experiments demonstrated that there was considerable "anticipation" effect in that variables, for example, heart rate, frequently changed at the point the mouthpiece was presented and before smoke was presented. In all studies, therefore, we accepted as control values those taken just before the subject accepted the mouthpiece. Moreover, the mouthpiece was routinely presented 2 minutes before the substance was ignited. Under the conditions of the experiments, 200 mg of material were ignited every 5 minutes, and the smoke concentration received was

TABLE I

CHARACTERISTICS OF SUBJECT POPULATION

Single Blind

Drug Order	Group	Age (Years)	Weight (Kg)	Education (Years of schooling)	Occupation
P — M	Non-experienced Males	51	83.0	9	Insurance
		29	80.0	9	Railroad worker
		49	97.0	15	Manager
		32	70.5	19	Graduate student
P — M	Experienced Males	23	63.5	9	Jeweller
		23	70.0	15	Student
		24	71.5	10	Labourer
		32	81.5	15	Computer Programmer
P — M	Non-experienced Females	25	52.8	16	Practical nurse
		28	66.5	12	Receptionist
		25	58.0	18	University instructor
		37	96.4	9	Housewife
P — M	Experienced Females	30	58.1	17	Housewife
		25	53.5	12	Keypunch Operator
		25	55.7	12	Caretaker
		25	51.0	16	Housewife
			Double Blind		
P — P	Experienced Males	22	60.5	13	Warehouse Manager
		27	64.5	12	Office clerk
P — P	Experienced Females	29	58.2	15	Nurse/housewife
		41	61.4	15	Nurse/housewife
M — P	Experienced Males	29	88.5	14	Mechanic
		31	80.0	12	Manager
M — P	Experienced Females	25	51.0	12	Nurse/housewife
		21	66.8	12	Secretary
P — M	Experienced Males	24	82.0	11	Plumber
		23	79.5	13	Autopaint mixer
P — M	Experienced Females	23	44.3	12	Housewife
		23	63.4	16	Teacher

P = Placebo M = Marihuana

highest immediately after each ignition. Continuous recording established that there was considerable variability in physiological measurements *during* the period of smoking either placebo or marihuana, and that this appeared to be related to the concentration of smoke. Accordingly all data collected *during* smoke inhalation were rejected. "Test" values were taken as those recorded 3 and 20 minutes respectively after termination of smoking. (These times were selected since during the period between 3 and 20 minutes, wrist cuff occlusion, Valsalva manoeuvre and cold pressor test were being done.) The greater of these test values was taken as representing the maximal change produced.

As was the case with the pilot experiments, no subject became intoxicated in response to placebo. Moreover, subjects showed no consistent changes in heart rate, arterial blood pressure or venous pressure following administration of placebo. In general, "dp/dt" tended to increase (not statistically significant), but there were no consistent changes in blood flow, vascular resistance or venous compliance. The placebo responses to the Valsalva manoeuvre and cold pressor test were unchanged from control.

Marihuana produced intoxication, as judged subjectively, or as judged by the experimenters, in 3 out of 8 non-experienced subjects. In the case of non-experienced subjects, intoxication was considered slight. Slight intoxication was present in one experienced subject, heavy intoxication in another, and moderate intoxication in the rest. Even though five non-experienced subjects exhibited no intoxication, they nevertheless all exhibited an increase in heart rate (Figure 2).

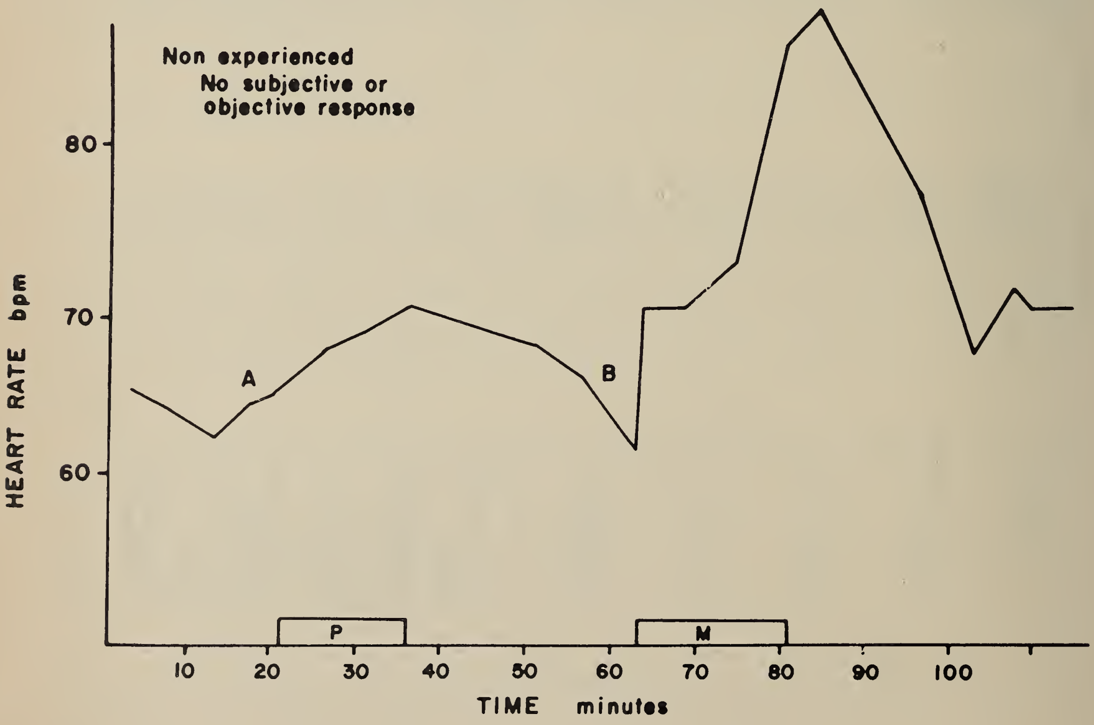

Figure 2. *Heart rate response in non-intoxicated subject following placebo (P) and marihuana (M). A and B respectively indicate the points at which the Fink valve was presented to the subject.*

Differences in the response to marihuana relative to placebo were examined by comparing pre-post smoking differences (marihuana) with pre-post smoking differences (placebo). Using this comparison, non-experienced subjects showed statistically significant increases in heart rate ($p<0.01$) and venous pressure ($p<0.02$). Experienced subjects showed statistically significant increases in heart rate only ($p<0.001$); the increase in venous pressure was not significant ($p<0.1$). When all subjects – experienced and non-experienced – were grouped together, there were statistically significant increases in heart rate ($p<0.001$) and venous pressure ($p<0.02$). Changes in other variables were not statistically significant.

The magnitude of responses to marihuana in experienced subjects tended to be greater than in non-experienced subjects, but a statistically significant "experience" difference was noted only in the case of heart rate ($p<0.02$) and "dp/dt" ($p<0.01$). There were no statistically significant "sex" differences in any variable. However, three of four experienced males exhibited a decrease in limb flow associated with an increase in resistance. The remaining experienced male, and three of four experienced females showed an increase in limb flow associated with a decrease in resistance. (Data were lost on the remaining female due to technical difficulties.)

Figure 3 shows responses in an experienced male subject to placebo and to marihuana. In this subject, as in all subjects who showed an increase in heart rate and "dp/dt", "dp/dt" was still increasing after heart rate had plateaued (or in some cases was decreasing).

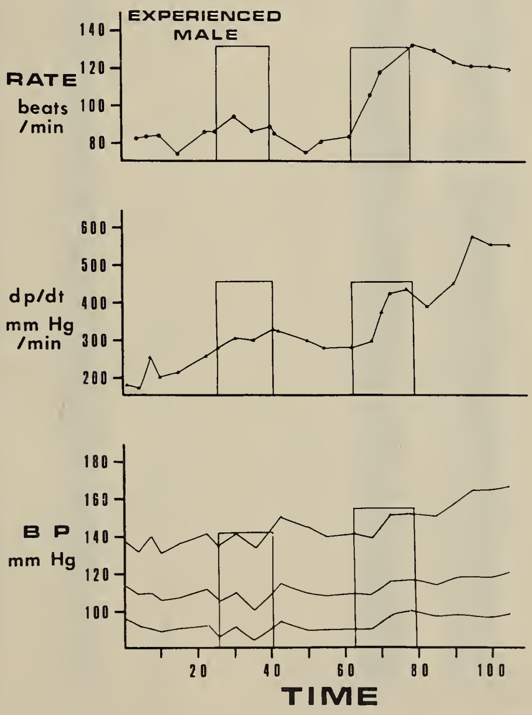

Figure 3. *Response to placebo (first block) and marihuana (second block) in an experienced male subject (single blind). Data obtained during the actual period of administration may be unreliable due to irritant effect of the smoke. The blood pressure data are those for systolic, mean and diastolic pressures respectively. Note that "dp/dt" is still increasing after marihuana at a time when heart rate is stable or decreasing.*

Figure 4 shows responses in two experienced male subjects to marihuana and to placebo. In response to marihuana, both subjects exhibited similar changes in heart rate, "dp/dt" and blood pressure. However, the first subject exhibited a decrease in forearm flow associated with an increase in calculated resistance, while the second subject exhibited decreased flow and increased peripheral resistance in parallel with intoxication. Neither type of response was associated with the presence of pain.

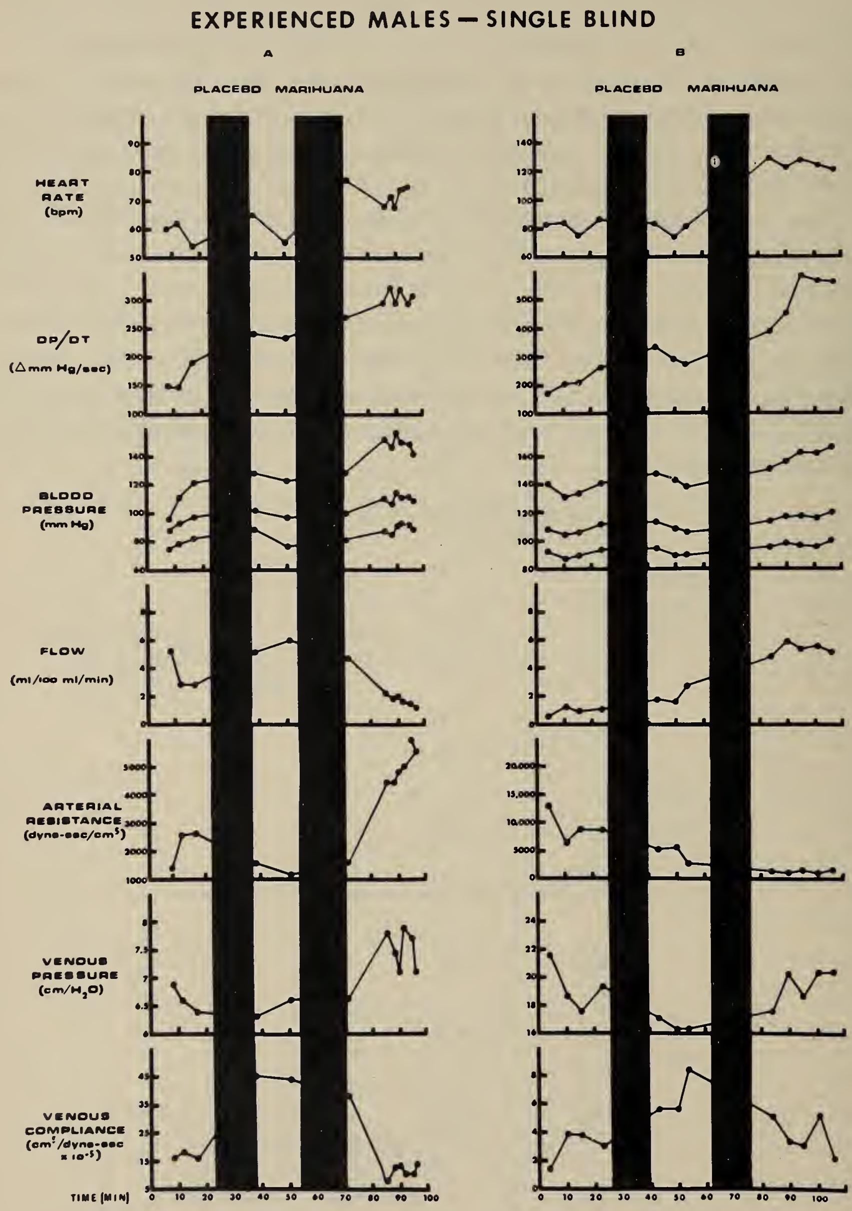

Figure 4. *Comparison of response to placebo (first block) and to marihuana (second block) in each of two experienced males (A and B) under single blind conditions. Note that in response to marihuana one subject shows decrease in forearm blood flow while the other subject exhibits an increase.*

Double Blind Experiments

The previous experiments convinced us that double blind experiments could be done safely. The experiments also indicated that the greatest amount of information would be obtained with experienced subjects.

The ages and weights of these 12 subjects are given in Table I. As a group, all female subjects were similar; the male subjects were also similar, one with the other, with the exception that the two males who received placebo followed by placebo were considerably lighter in weight than other males. All of the females used alcohol in moderation, but one of the male subjects averaged 18 bottles of beer and 40 ounces of alcohol per week. All subjects used marihuana or hashish at least once a month. One male and four females had used hallucinogens previously.

Of the 12 subjects, 4 received placebo on both occasions. In each instance, neither administration of placebo produced intoxication, and there was no difference in physiological response between the two placebo administrations. This served as an effective "time control" to establish that the effects noted in the single blind experiments were indeed attributable to marihuana.

All 8 subjects who received marihuana became intoxicated.

Four subjects received marihuana and placebo in that order. Although the marihuana response was clearly different from placebo, these "marihuana" data could be compared only to the control period and not to the placebo period since there was a possibility that the response to placebo was contaminated by the previous marihuana.

Although the experimenters did not talk to the subjects during the procedure, they were able to identify with 90% accuracy from the physiological data which substance was received on which occasion. This identification was also possible solely from records by those who had not been in the experimental room. These observations indicate that a double blind cannot be maintained when physiological records are available to the investigator. As a consequence, the data for the four subjects who received placebo and marihuana in that order (same order as single blind experiments) were grouped with the data from the single blind experiments.

Figure 5 shows the average percentage change in the various parameters of each subgroup (single blind plus double blind) in response to placebo and marihuana.

The pattern of response to marihuana is one of increases in heart rate, dp/dt, arterial blood pressure and venous blood pressure, which are more marked in experienced males. In the experienced male subjects the trend was toward an increase in peripheral resistance which was not significant ($p<0.1$). In contrast, females tended to show a decrease in peripheral resistance, and an increase in flow. These changes were statistically significant. There were no specific effects on "skin" or "muscle" blood flow as indexed by wrist cuff occlusion.

Induced Changes in Autonomic Tone

The apparent temporal dissociation of positive chronotropic effects and positive inotropic effects (as judged by "dp/dt") suggests a vagal inhibitory effect. Two tests were done at a point in time when this vagal inhibitory effect might have been expected to be present: the Valsalva manoeuvre and the cold pressor test. In retro-

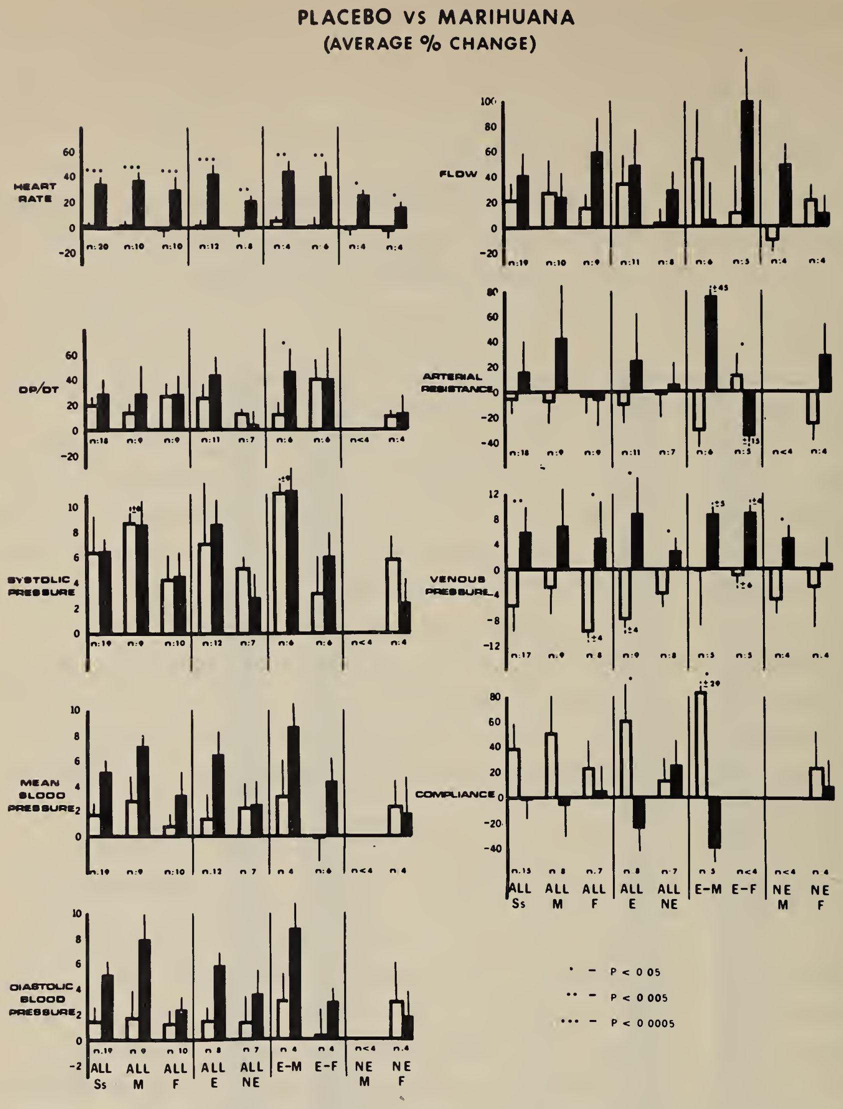

Figure 5. *Comparison of response (± % change from control (0)) to placebo (open bars) and to marihuana (closed bars) for all subjects (single blind plus double blind). Lines indicate standard deviation of mean. Only 4 non-experienced males and 4 non-experienced females were used as subjects. Some data from one subject in each group was lost due to technical difficulties. In such cases the notation N<4 is used to indicate statistical analysis was not possible. Ss = subjects; M = males; F = females; E = experienced; NE = non-experienced.*

spect, it is unfortunate that these were not done repetitively during intoxication to index any change.

Figure 6 shows a representative response to the Valsalva manoeuvre under placebo and marihuana conditions. When marihuana has been presented, the initial heart rate and blood pressure are slightly elevated, but the fall in blood pressure is greater, and the "overshoot" less, both suggesting some lessening of sympathetic tone. Moreover, the final vagal slowing is less marked, and while this may be a function of the greater initial heart rate, it may also reflect some vagal inhibition. The statistical values given are those derived from the 12 subjects in whom this procedure was done.

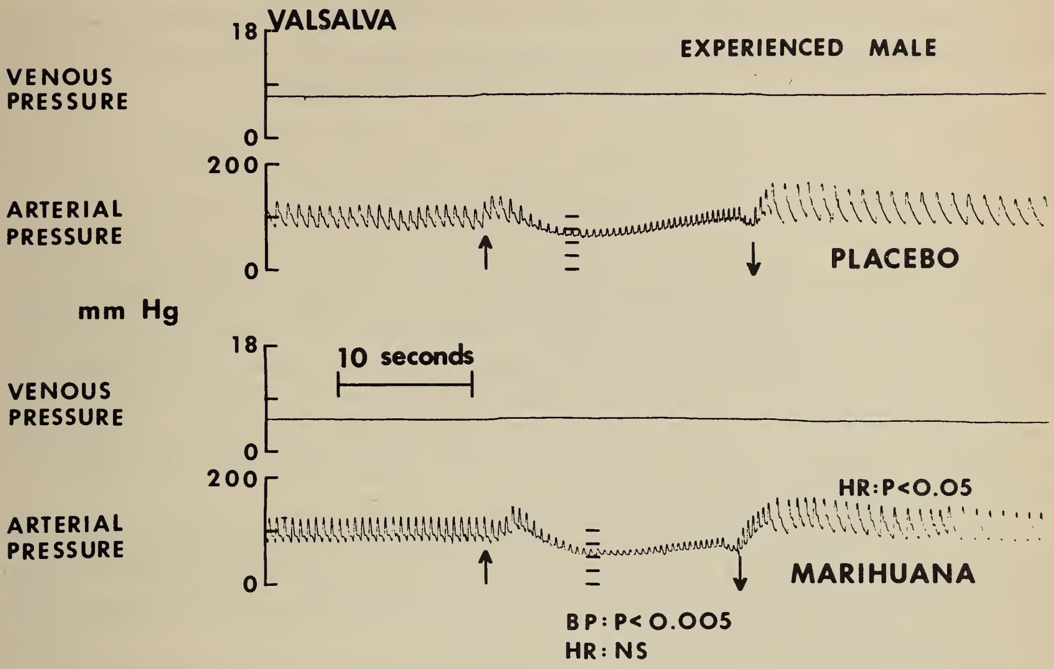

Figure 6. *Representative Valsalva response in experienced male following placebo (upper panel) and following marihuana (lower panel). Arrows indicate beginning and end of manoeuvre. Pooled data from all subjects were used to obtain the statistical values shown.*

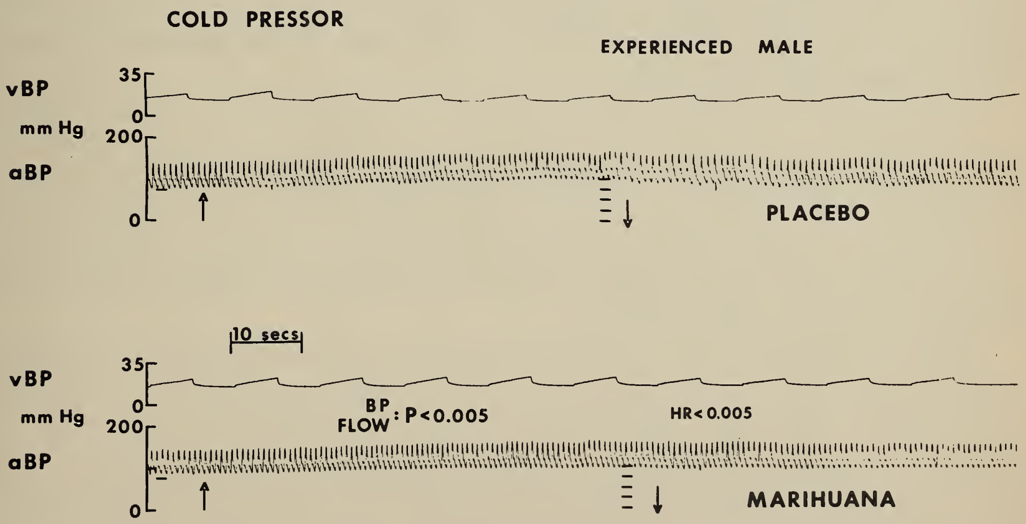

Figure 7. *Representative cold pressor response in experienced male following placebo (upper panel) and following marihuana (lower panel). Arrows indicate beginning and end of immersion of opposite hand in water at 1 degree C. vBP = venous pressure; aBP = arterial pressure. Pooled data from all subjects were used to obtain the statistical values shown.*

Figure 7 shows the response to a cold pressor test. After marihuana the initial heart rate and arterial pressures are slightly elevated. Immersion of the hand in ice water produced less of an increase than under placebo conditions. The quantitatively smaller response of the blood pressure (but not heart rate) was statistically significant in the 12 subjects in whom this manoeuvre was done. The decrease in forearm blood flow during cold pressor was also significantly less following marihuana. Likewise, the post-cold pressor bradycardia was significantly reduced.

DISCUSSION

The present study on the cardiovascular effects of marihuana differs from previous studies in comparing responses not only in experienced and non-experienced individuals but also in both females and males. It extends previous observations by comparing data obtained under single blind conditions with those obtained under double blind conditions. In general, this study, with its added measure of control of set, setting, and protocol, augments previous, less controlled studies, and lends support to the conclusions reached in such studies.

The cardiovascular responses observed are consistent enough that the investigators were able to "break" a double blind code simply by inspecting the physiological data. At the same time, the magnitude of the cardiovascular response to intoxicating doses, while statistically significant, is not marked.

The difference in subjective intoxication between experienced subjects (all of whom became intoxicated) and inexperienced subjects (37% of whom became intoxicated) support anecdotal comments that experienced individuals more easily become "high". It is of interest that non-experienced subjects who do not become intoxicated (see Figure 4) may, nevertheless, exhibit characteristic cardiovascular responses. To our knowledge, this is the first time that it has been demonstrated that at least some of the physiological effects of marihuana are not secondary to excitement associated with intoxication.

In general, the cardiovascular responses were quantitatively greater, at a fixed dose of marihuana, in experienced individuals than in non-experienced individuals. However, this difference was statistically significant only in the case of heart rate and "dp/dt". Weil *et al.*[11] also found a greater increase in the heart rates of chronic users than those of naïve subjects. While Renault *et al.*[13] found no such difference, their particular study was characterized by small numbers of subjects, inadequately defined according to drug experience.

Other investigators have reported tolerance to behavioural effects of cannabis in animals in response to large doses given on a repetitive basis;[14] however, firm data which would extend such observations to man are lacking. Certainly, under the conditions of these experiments, there is no suggestion in our subjects of the presence of pharmacological tolerance to the cardiovascular effects of marihuana. If such tolerance were present, the response would be expected to be less in the case of the experienced person.

We are unable to document a sex difference in the cardiovascular response to marihuana. Males in general tended to exhibit more marked cardiovascular responses than females. However, these quantitative differences were not statistically significant. The data relating to effects on peripheral blood flow might be taken to indicate possible qualitative differences between the sexes, but the variability is such that inter-subject differences independent of sex appears more likely.

Tachycardia was the most consistent cardiovascular response to marihuana. The increased heart rate could be due to direct or indirect sympathetic stimulation, vagal inhibition, or a combination of both. The fact that it occurs in subjects who do not become intoxicated casts some doubt on psychogenically-mediated increases in sympathetic tone as a mechanism.

The temporal dissociation, early in the intoxication, between "dp/dt" and tachycardia is similar to that which one sees with atropine and suggests vagal inhibition. This suggestion is supported by data obtained with the Valsalva and cold pressor tests. The Valsalva manoeuvres were less controlled in our experiments than we would ideally have liked. However, while the subjects did not expire against a known resistance, the duration of the manoeuvre was standardized and all subjects had preliminary practice runs to ensure consistency. An analysis of the response to the Valsalva manoeuvre after marihuana shows that the bradycardia which occurs after release of breath holding is less marked than under placebo conditions. (The bradycardia can be blocked by anticholinergic agents such as propantheline and is thus believed to occur by a vagal mechanism.)[15] While this observation can be taken to support a thesis of vagal inhibition, it must also be noted that the basal heart rate, at the beginning of the Valsalva manoeuvre, was greater after marihuana than after placebo. The bradycardia which occurs in the cold pressor test on removal of the cold stimulus was also less under marihuana conditions than under placebo conditions.

Taken collectively, these data suggest that the early tachycardia in response to marihuana may be due in part to vagal inhibition. Additional evidence for an atropine-like action of cannabis in man is supplied by Renault, Schuster, Heinrich *et al.*[13] They report a suppression of sinus arrhythmia by cannabis. Since this arrhythmia is mediated by the vagus, they suggest marihuana may alter normal vagal tone. In retrospect, in attempting to dissociate sympathetic and vagal factors, it is unfortunate in our experiments, that the Valsalva manoeuvre was not done repetitively at a later time when increases in heart rate were temporally associated with increases in "dp/dt".

While there are indices of increased sympathetic tone during this stable tachycardia associated with intoxication (for example, increased "dp/dt", increased venous pressure, and increased peripheral resistance (particularly in male subjects)), there are also indications of decreased reflex sympathetic responses. In our experiments, the hypotensive phase of the Valsalva manoeuvre was more marked after marihuana than after placebo. This type of response is similar to that seen following surgical[16] and chemical[15-17] interference with efferent sympathetics and to that observed in certain pathological conditions such as tabes dorsalis[15] or familial dysautonomia[18] where interference is believed to occur on the afferent side of the baroreceptor reflex arc. The blood pressure "overshoot" which occurs after the Valsalva manoeuvre was also less marked after marihuana than after placebo. (It has generally been accepted that this overshoot is a result of restored cardiac output forced into a blood reservoir still constricted from the hypotensive period.)[15,19,20,] Again, this diminution in response after marihuana suggests some disturbance in homeostatic mechanisms. Similar inferences could be drawn from the observation that the rise in pressure and decrease in flow occurring with the cold pressor test is less marked after marihuana than after placebo. (However, the baseline blood pressure at initiation of the cold pressor test was also greater.)

These latter data are consistent with those in a recent paper by Beaconsfield, Ginsburg and Rainsbury[4] and support their suggestion of impaired reflex responses following cannabis. Similarly, in studies on anesthetized dogs Dewey, Yance, Harris *et al.*[21] have shown that the pressor response to bilateral carotid occlusion is blocked by 1.0 mg/Kg of Δ^9-THC.

Although it is not emphasized in the literature, other authors have noted an increase in blood pressure in supine subjects in response to marihuana.[1] The small rise in blood pressure which we observed in our experiments was most likely due to an increase in cardiac output. Certainly increased vasoconstriction did not play a significant role since the increase in blood pressure occurred even in those subjects who showed a decrease in regional vascular resistance.

Beaconsfield *et al.*[4] found significant increases in peripheral blood flow in non-experienced males in response to marihuana; Weiss *et al.*[3] studied only experienced males and reported significant increases in forearm flow in the supine but not the upright position. In contrast, in our study there was a statistically significant increase only in the case of experienced females ($p<0.05$). When wrist cuff occlusion was used as an index of partitioning between skin and muscle blood flow, we were unable to document consistent marihuana effects. Similarly reactive hyperaemia was not influenced.

Further experimentation is necessary to establish precisely the effects of marihuana on the peripheral circulation. The general pattern, however is one of peripheral cardiovascular effects being even less marked than effects on the heart itself.

In summary, this study has examined under well controlled circumstances the cardiovascular responses to marihuana in man. While subjects with existing heart disease have not been studied, no data have emerged which would indicate that acute administration of this substance presents a significant hazard to the cardiovascular system. If the data which suggest a muting of reflex cardiovascular responses can be corroborated, however, one might have some apprehension that, in an emergency, an intoxicated individual's reflex vascular responses may be reduced to a clinically significant degree.

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Some Methodological Considerations in Research on Joint Action

John A. Carpenter, Ph.D.

ABSTRACT There is little agreement on definitions which describe the combined actions of chemical agents, such as synergism, antagonism, etc. In addition, these terms do not specify operations by which agreement between the terms and observed results of drug combinations can be decided. Unambiguous mathematical statements have been sought (based on significant pharmacological concepts) to describe the results of combining chemical agents. Existing models were inadequate because they were restricted to quantal data and to monotonic relations. Simultaneous with model development, data on two drugs, alcohol and meprobamate, were gathered in five experiments to establish the dose-response function for each drug alone, to study combinations in which meprobamate was administered chronically and acutely. The data of the experiments do not provide an adequate test of the validity of the models, because the doses do not discriminate between the models that could fit the results of the meprobamate-alcohol combination. The behaviour in response to the doses of these two drugs in combination could not be characterized by the existing terminology (additive, synergistic, etc.). The individual and combined functions were too complicated. Blood levels of meprobamate were found to be related to the presence of alcohol and the results suggest that the reverse may also be true.

The material presented here, based on the joint research of Drs. Carpenter, Gibbins, Marshman, Ashford and Cobby,[1] concerns the conditions necessary for the interpretation of the results of studies of drug combinations and suggests how data are to be gathered in order to satisfy these conditions. These remarks are a brief overview of our analyses of alcohol and meprobamate, the results of which will be published in the spring of 1975.

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If we compare definitions of terminology used to express the result of chemical combinations on biological material, we find a certain amount of agreement. Among the various terms, synergism is the focus of most discontent. Veldstra,[2] Jawetz and Gunnison,[3] Tammes,[4] Hewlett,[5] and Bliss,[6] all of whom represent different disciplines, define synergism similarly, essentially the same as Webster. Hewlett[5] captures the flavor of the idea very well. Synergism is reserved for "situations in which the mixture exceeds the 'sum' of the effects of the separate constitutents — when the use of the two or more components together gives, as it were, a special bonus of biological activity".

The agreement seen among these workers is not universal, however. For example, Goodman and Gilman[7] reserve synergism for a special case, that of one active agent in combination with an inactive agent. According to Macgregor,[8] "synergistic action of drugs may result from summated actions. . .", while potentiation refers to actions that are "greater than the sum of the parts". It has also been used in other ways, e.g., Wallgren and Barry.[9]

It is apparent from reviewing the meanings of synergism that terms are required to express three classes of result: additive, greater than additive, and less than additive, with respect to the activity of the agents considered singly. It would seem that if we could determine additivity, the other two cases would fall into place. It turns out that additivity, meaning algebraic summation, is not easy to determine. Without being able to do even this, one may argue endlessly about the terms. The difficulty lies in the fact that the terms synergism, potentiation, antagonism, additive, and so on, do not specify operations by which agreement with empirical events can be decided in all cases. Consequently the utility of these terms is seriously limited. In recognition of this limitation, unambiguous mathematical statements have been sought to describe the possible results of combining chemical agents. These are the mathematical models of the joint action of agents, such as drugs, pesticides, antibiotics and so on.

The development of joint-action models has a long history, beginning with Bliss.[6] These models require that enough different doses of each chemical agent be used to express the relation between response and dose. For Bliss[6] this meant that each chemical agent had to have a linear dose-response function. For the most recent models[10] there is no restriction on shape: linear, nonlinear, and nonmonotonic, and in any combination, and for any number of drugs. The minimum number of administered doses necessary to express a nonlinear relation is four, and certainty increases as the number of doses increases especially for those drugs possessing nonmonotonic functions. When circulating levels of a drug can be substituted for administered dose, the relation is strengthened because more points representing the drug are available. The relation between circulating drug and administered dose depends on the method of administration: with oral administration the relation need not be monotonic, depending on a number of factors. Once we begin to consider combinations of agents that have nonlinear dose-response functions the difficulty of determining additivity is apparent and may be beyond our ability, without the aid of mathematics. If additivity, conceptually the most obvious, cannot be ascertained, we may question whether any other kind of relation can be understood.

Two other developments have occurred in the genesis of mathematical models of joint action. One is that any conclusion as to the outcome of a combination of chemical agents should remain invariant under transformation of the doses of each drug;[11] that is, if a combination appears to be synergistic (Webster's definition) it cannot be made additive by using a transformation, e.g., dose instead of log-dose. The other development extends the application of models in a necessary direction. Because the early models arose from a need to increase the toxicity of pesticides, the responses were quantal (all-or-none) and it was difficult, if not impossible, to extend their use to quantitative responses, such as sleeping time or the kind of data to be expected from human subjects.

A set of goals may be stated for mathematical models. We would like them to provide: (1) a description of the nature of the action of single drugs; (2) a comprehensive classification of the results of drug combinations; (3) a set of operations for deciding the outcome of combinations, including the minimum number of doses of each drug and the approximate values of their doses required to discriminate among the probable outcomes; and (4) the capability of predicting all possible results of a combination from a knowledge of each drug acting alone. Considering the number of drug combinations possible, the utility of point (4) alone would be great, since it leads to the possibility of screening combinations by computer simulation.

Two things are necessary to produce models that satisfy these goals. One is that mathematical representation of a small number of concepts concerned with the action of drugs must be found; the other is that the mathematical models must be tested on real data.

Having decided among ourselves that mathematical models were necessary, that existing ones were inadequate for our purposes, and that there was little if any data on which to test models, we began simultaneously model development (Ashford and Cobby[1]) and data gathering (Carpenter, Gibbins and Marshman[1]). The special features of the new models were that they be able to handle quantitative response-data and nonmonotonic dose-response functions. Behavioral data were to be obtained from at least five administered doses of each of two drugs and blood levels of each drug were to be determined.

METHOD

Five experiments were carried out. Experiment 1 turned out to be rehearsal for the others. Experiment 2 established the dose-response function of meprobamate, using the following doses: 0, 5, 10, 15, 20, 25, 30 mg/kg. Experiment 3 was to establish the dose-response function of alcohol in the doses: 0, .25, .50, .75, 1.00 g/kg. In Experiment 5 the two drugs were administered together in factorial combinations of dose. In this experiment the doses of meprobamate were altered to 0, 7, 14, 21, 28 mg/kg to cover approximately the same range as Experiment 2 but to restrict the number of doses to five. In Experiments 2 and 5 meprobamate was administered as a single dose one hour before the administration of alcohol. Experiment 4 differs in that the same doses of meprobamate were divided into thirds and spread throughout the day. Subjects were maintained on a single dose for 12 days, with alcohol administration starting on day 8.

Drugs

Alcohol was chosen because of our experience with its use in laboratory experiments and its social significance. Meprobamate was chosen because of its record of safety[12] and because of its extensive use.[13] Because Zirkle *et al.*[14] had reported untoward effects with a combination of chronically administered meprobamate and alcohol, and given our lack of experience with combinations, we felt that a more potent drug might be hazardous in combination with alcohol. Administration of the drug followed the same patterns in Experiments 1, 2, 3 and 5: ten tablets composed of 0, 100, 200 or 400 mg of meprobamate regardless of dose, were consumed orally. Four tablets were administered on each of the three daily occasions in Experiment 4. Alcohol was taken 1 hour after meprobamate as 25% solution with orange juice in Experiments 1, 2, 3 and 5; in Experiment 4 the alcohol was consumed a half hour after the second daily administration of meprobamate. One hour was allowed for drinking the alcohol in all experiments. In all experiments it appeared to the subjects that both drugs were administered on all occasions.

Design

In Experiments 2 and 3, 8 subjects were assigned to a single dose of meprobamate and alcohol resulting in 56 and 40 subjects in the two experiments respectively. In Experiment 4 each subject was confined to a single dose of meprobamate for the duration of the experiment and received all doses of alcohol, a different one on each experimental day. The positions of the drugs were reversed in Experiment 5. Each subject was confined to a single dose of alcohol and received a different dose of meprobamate on each experimental day. In both experiments there were 25 subjects, 5 for each dose of the unchanging drug. Nine samples of behavior were obtained at half-hour intervals following alcohol ingestion in all experiments. In Experiment 4 one pre-alcohol sample of behavior was obtained. In all others two were obtained.

Blood Samples

Blood samples were taken as frequently as circumstances would permit. In Experiments 2 and 3, 11 blood samples were taken at half-hour intervals beginning with the consumption of alcohol, and one sample was obtained at the time of meprobamate administration. In Experiment 5, because the subjects were used five times, 8 blood samples per day were obtained, one at meprobamate administration, one at alcohol administration, 4 at half-hourly intervals following alcohol and 2 at hourly intervals after that. In Experiment 4, where subjects participated for 12 days, it was necessary to reduce the number of blood samples to 3 for the first 7 days and 4 for the last 5 days. The samples were taken at the times of meprobamate administration and one hour after alcohol. The determination of meprobamate and ethanol levels in the blood was made by gas chromatography.

Subjects

One hundred and fifty-eight men were used as subjects in these experiments. The ages ranged from 21 to 49, the average being 23 years. The subjects of Experiments 2, 3, 4 and 5 were obtained from colleges around Toronto or were unemployed Canadian immigrants. Subjects were screened medically and psychiatrically.

Behavior

The behavioral task is described as a step-input tracking task presented to the subject by the "Stressalyzer."[15] It is primarily a hand-eye coordination problem, involving moving a pointer from a lighted target to one of four others as they appear in random order. The pointer was activated by an automobile steering wheel which moved the pointer in a direction opposite to the movement of the wheel. The task is quite difficult to perform. It provides a number of simple measures, such as reaction latency, targets missed, errors (errors of direction of wheel movement).

RESULTS

The results presented here are chosen to illustrate some of the issues raised in the introduction. They are purposely sampled and do not represent the full set of data.

Blood Meprobamate Concentration

Factors influencing the concentration of meprobamate in the blood (BMC) are of some interest since they determine the extent to which the administered dose may be used to represent the effective dose of the drug. This assumes that effective dose is a simple function of blood level.

The chief outcome of Experiment 1 was that it served as a rehearsal for the later experiments. Some results are interesting in spite of an unknown degree of bias. In Experiment 1 (meprobamate dose = 25 mg/kg) circumstances led to the use of two age groups of subjects. BMC as a function of time for older and younger subjects is shown in Figure 1. The 6 older subjects (27 years and above) have a more rapid absorption of meprobamate than the 5 younger subjects (26 years or younger). The Age by Time interaction is significant ($p<.01$). As a matter of fact the analysis shows that BMC is a different curvilinear function of time (Time Linear X Age, $p<.0005$, Time Quadratic X Age not significant but too large to be ignored) for the two groups. The older subjects reached near peak BMC two hours before the younger subjects.

Figure 2 shows BMC for 6 subjects of Experiment 1 who received meprobamate at 25 mg/kg on two occasions and alcohol at about 1.0 g/kg on one of those occasions. Analysis shows the Time Linear X Alcohol and Time Quadratic X Alcohol interactions to be highly significant ($p<.005$ in both cases). When alcohol was administered, BMC was lower than when no alcohol was consumed. Examination of Periods 3, 4 and 5 (not in Figure 2) shows that BMC was higher when alcohol was given than when it was not, in contrast with later periods. Although the results of Experiment 1 must be taken with caution because these analyses represent a patchwork of condition for which the experiment was not designed, it is apparent that some factors other than meprobamate dose itself had a strong influence on BMC.

In Experiment 5, experimental conditions were under excellent control: uniform subject sample, rigidly controlled doses, well-trained crew of technicians and

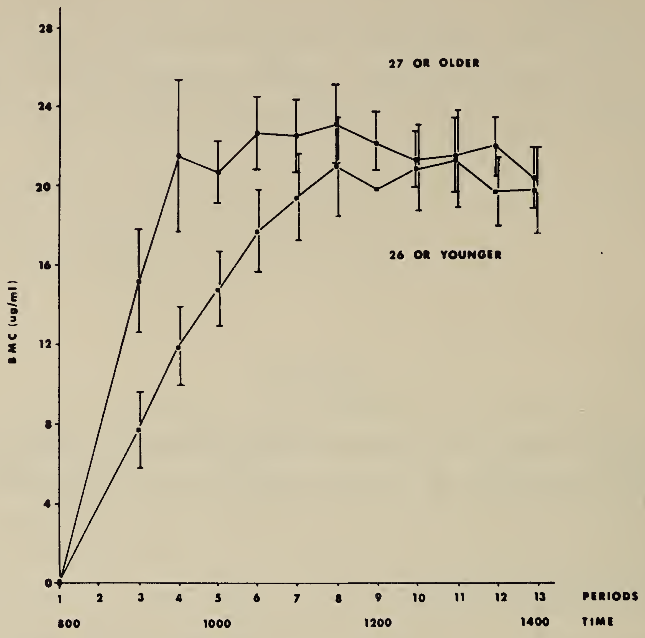

Figure 1. *Blood Meprobamate Concentration as a Function of Time after Administration of 25 mg/kg Meprobamate for Older and Younger Subjects in Experiment 1.*

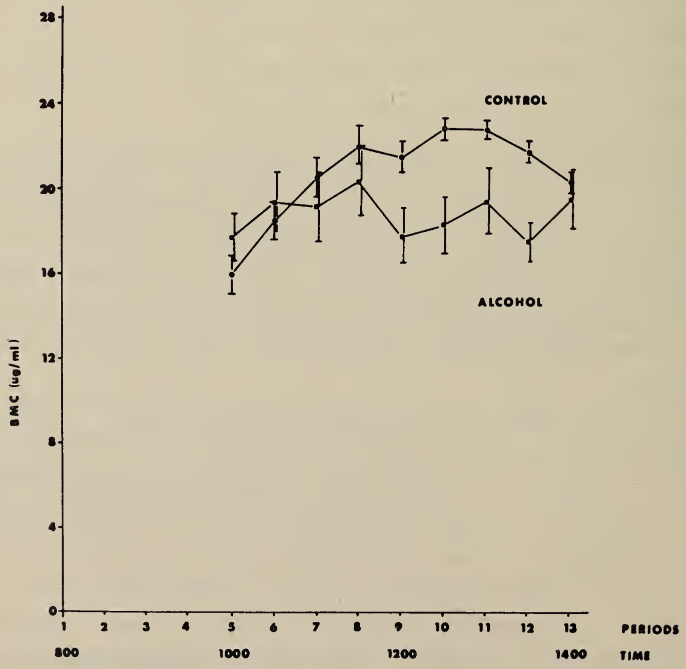

Figure 2. *Blood Meprobamate Concentration (25 mg/kg Meprobamate) for Subjects With and Without Approximately 1.0 g/kg Alcohol (Experiment 1).*

assistants. Figure 3 shows BMC as a function of both meprobamate and alcohol dose at Period 5, 2 hours after the administration of meprobamate and 1 hour after beginning to drink. It is apparent that the dose of alcohol has influenced BMC. Figure 4 shows BMC one half hour after Figure 3 at Period 6 and Figure 5 shows BMC 4.5 hours after beginning to drink at Period 12. The BMC from the 6 post-drinking blood samples, in conjunction with the doses of meprobamate and alcohol (the Time X Meprobamate X Alcohol interaction), have significant components ($p<.05$, $p<.025$) that indicate that these are complex surfaces changing over time. Time X Alcohol is also significant, the degree of significance depending on how the error terms are chosen (the more conservative give $p<.05$, the other, $p<.005$). The absence of influence of alcohol on the 7 mg/kg meprobamate dose should be observed, because different subjects were used at each dose of alcohol. If the corrugated surfaces at higher doses of meprobamate were due to failure to assign subjects randomly to doses of alcohol, the same corrugated effect would be expected at the 7 mg/kg dose of meprobamate.

The interaction of time, meprobamate and alcohol on BMC was also seen in Experiment 4. The surfaces look quite different with smaller differences in BMC, but with better levels of significance due to error variations being about half of those in Experiment 5. The influence of alcohol on BMC would be expected to be smaller in Experiment 4 because BMC levels had been established by a week or more of chronic administration of meprobamate and only a 1/3 dose was administered just prior to alcohol ingestion. *In vitro* experiments in response to these results show that more meprobamate goes into solution when alcohol (as compared to water) is added simultaneously with meprobamate to gastric juice. If the water or alcohol is added one hour after the meprobamate the effect is a great deal stronger. It is apparent from these results that alcohol as administered in these experiments can influence BMC levels, possibly by absorption.

One further point on the influence of alcohol on meprobamate levels can be made from Experiment 4. It will be remembered that meprobamate was administered for 12 days at constant dosage for groups of subjects. On the eighth day alcohol administration at five doses was begun and carried through day 12. The question here is, did alcohol administration, begun on day 8, influence the BMC as compared to the BMC prior to day 8. Because BMC had reached maximum values by day 5, BMC for days 5, 6 and 7 were compared to days 8 to 12. The last blood sample of the day, 5.5 hours after beginning to drink, was used for this analysis because it was the only one that occupied the same relation to the dose of meprobamate throughout the 12 days and also occurred after alcohol administration. Figure 6 shows BMC as a function of meprobamate dose for alcohol and non-alcohol days. BMC for non-alcohol days is a curvilinear function of meprobamate doses, curvature being caused almost completely by the 28 mg/kg dose. The function for alcohol days is linear. The difference between the means at this point is only about 2.8 μg/ml. The overall interaction between doses of meprobamate and alcohol is significant ($p<.025$). Further analysis shows the Meprobamate Linear X Alcohol, and Meprobamate Quadratic X Alcohol terms to be significant ($p<.05$ and $p = .01$). This means that alcohol has modified the relation between administered dose of meprobamate and BMC. In other words the two functions have different shapes, resulting in higher BMC at the 28 mg/kg dose of meprobamate on alcohol days.

Blood Alcohol Concentration.

No influence of meprobamate dose on blood alcohol concentration (BAC) was detected in Experiments 1 and 4. Contradictory results were observed for Experiment 5 due to different analytical procedures adopted by Cobby and Ashford[1] on one hand and Carpenter[1] on the other. Both procedures are defensible. However, if there is a true influence it occurs at period 5, one hour after beginning to consume the alcohol. Figure 7 shows the surface for BAC as a function of both drugs at Period 5. The major effects appears at .75 and 1.0 g/kg primarily at 7 mg/kg meprobamate. The surfaces for successive blood samples become increasingly regular.

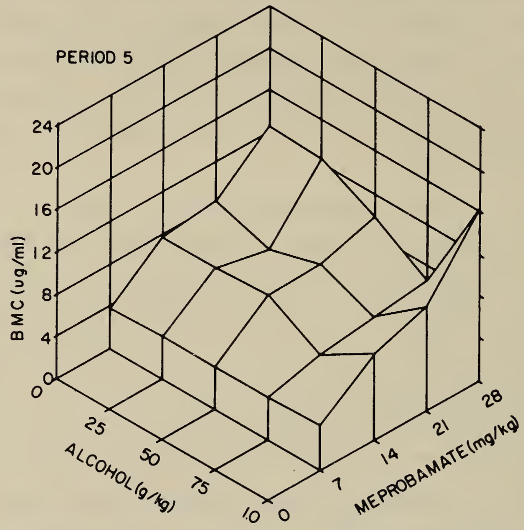

Figure 3. *Blood Meprobamate Concentration as a Function of Dose of Meprobamate and Alcohol One Hour after Beginning to Drink (Experiment 5).*

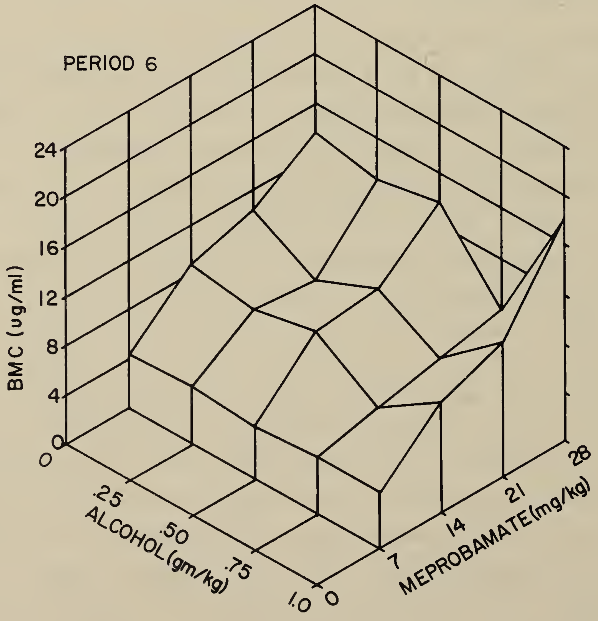

Figure 4. *Blood Meprobamate Concentration as a Function of Dose of Meprobamate and Alcohol 1.5 Hours after Beginning to Drink (Experiment 5).*

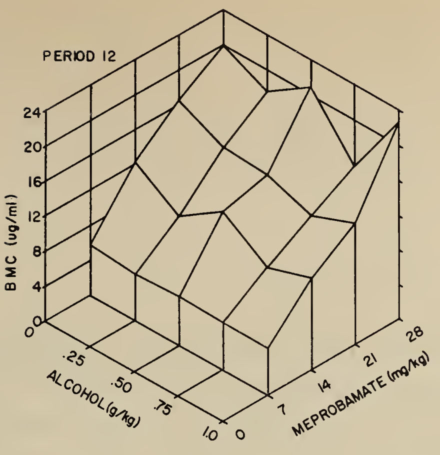

Figure 5. *Blood Meprobamate Concentration as a Function of Dose of Meprobamate and Alcohol 4.5 Hours after Beginning to Drink (Experiment 5).*

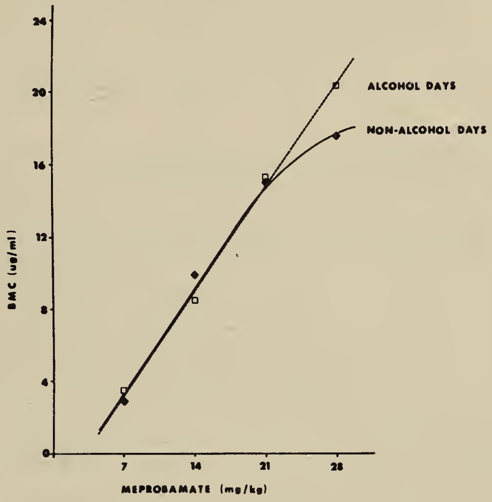

Figure 6. *The Relation between Blood Meprobamate Concentration and Meprobamate on Alcohol and Non-Alcohol Days (Experiment 4).*

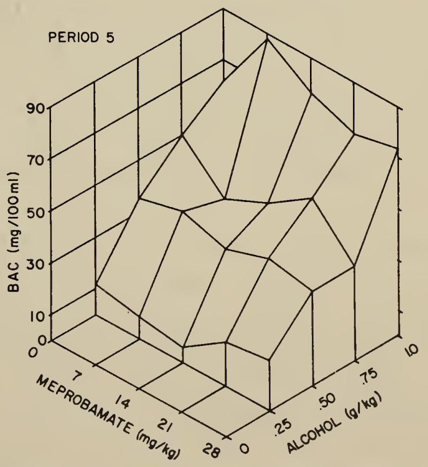

Figure 7. *Blood Alcohol Concentration as a Function of Dose of Alcohol and Meprobamate One Hour after Beginning to Drink (Experiment 5).*

In concluding this part, we would like to point out that the blood levels of either drug obtained at the zero dose of the other are quite reasonably regular as compared with those observed when active doses of the other drug were used. There is a good deal of suspicion that using orally administered dose as an equivalent of effective dose would be a hazardous procedure, at least for testing models of joint action.

Measures of Behavior

One argument was presented that nonmonotonic functions occur in drug experiments and that mathematical models had to have the capacity to handle them. Finney [16] cites an example of the inhibition of spore germination as a function of the log-deposit of tetramethylthiuram disulfide. The curve has two sharp changes of direction. The effect is attributed to potencies of the dissociated and undissociated molecule which occur in different proportions at different concentrations of the agent. In our own work nonmonotonic functions of behavior in relation to alcohol dose have been observed.[17, 18] These curves are U or inverted U-shaped, depending on the measures used. Let us look at the results obtained from the experiments under consideration here.

The purpose of Experiment 2 was to obtain a dose-response function for meprobamate. All behavioral results are corrected for the pre-drinking performance of each subject, on each experimental day. Figure 8 shows the relation between ST and the oral meprobamate dose. ST is defined as the product of Reaction Latency and Targets Missed plus Errors. It was chosen for presentation here because it exaggerates some results found for all other measures: Errors, Targets Missed, Targets Missed plus Errors and Reaction Latency. The curve of Figure 8 has two significant components of trend: the linear and fifth power components of meprobamate dose. The linear component has an F at $p<.025$ and represents 27% of the total variation attributable to meprobamate. The fifth power component has an F at $p<.001$, representing 65% of total variation in meprobamate doses. The two components together represent 92% of the variation produced by meprobamate. All measures made in this experiment (component measures of ST) show this same saw-toothed shape and all are significant but to a lesser degree. The curve says that there is a mild, general decrease in performance as meprobamate rises and a good deal of variation from dose to dose. The curve in Figure 8 includes the data from all 9 post-alcohol (zero dose in this experiment) periods (4 hours). The results are amazingly consistent at each period. We have to assume in the absence of other evidence that this curve represents the meprobamate effect, and it is definitely nonmonotonic.

In contrast to the meprobamate curve is the one for alcohol obtained from Experiment 3. Figure 9 presents these results for ST obtained from all 9 post-drinking periods. The curve is a straight line. The linear component of Alcohol is highly significant, $p<.0005$, and represents 95% of the variation attributable to the doses of alcohol. These results show much more change over the 9 post-alcohol periods, as would be expected from a rapidly absorbed and eliminated drug.

Here we may speculate about the difficulty of deciding the outcome, additive or otherwise, without the aid of mathematics, of a combination of two drugs that have curves of the type represented by meprobamate and alcohol.

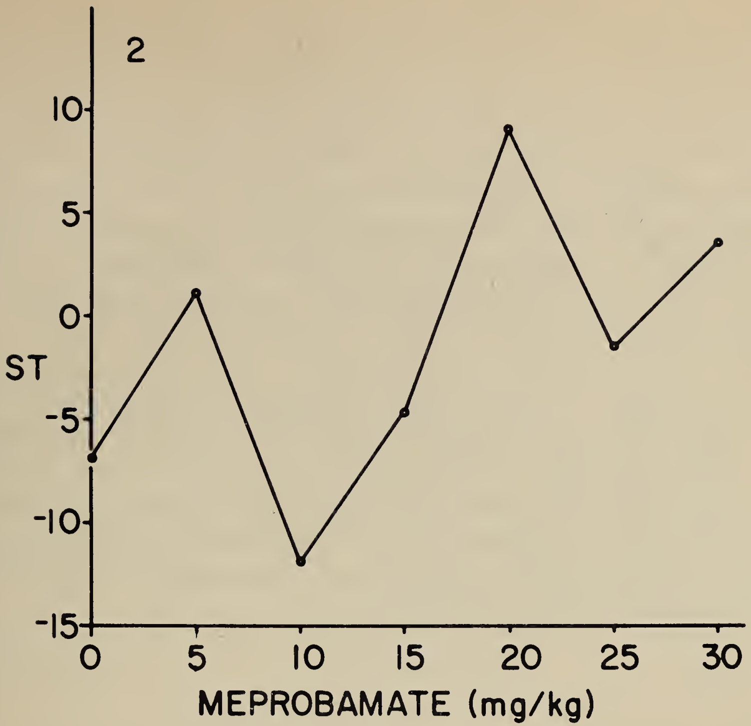

Figure 8. *The Dose-Response Function for the Behavioral Measure ST and Meprobamate Dose in Experiment 2.*

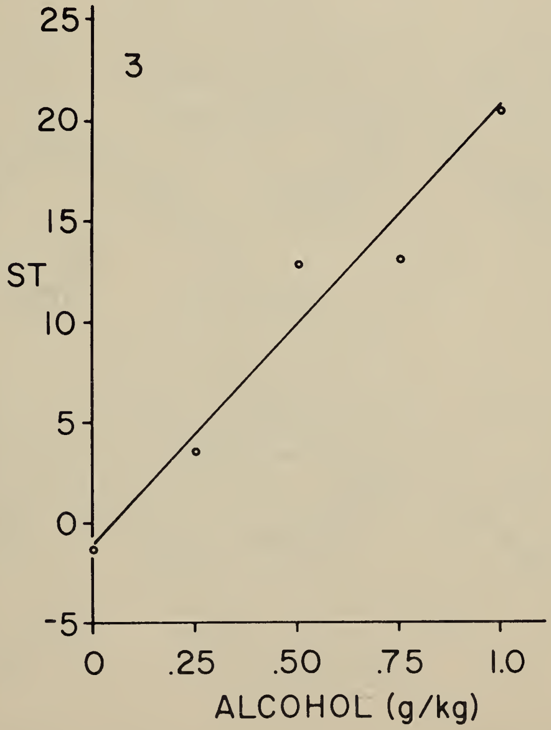

Figure 9. *The Dose-Response Function for the Behavioral Measure ST and Alcohol Dose in Experiment 3.*

Figures 10, 11, and 12 present the results of the combinations of the two drugs taken from Experiments 4 and 5 for successive post-alcohol periods. The upper curves are from Experiment 5. The proper comparison for Experiments 2 and 3 is Experiment 5 because meprobamate appeared to be or was administered acutely in all three. The appropriate term from the analysis of variance is the PMA interaction (Periods X Meprobamate X Alcohol) which is simply variation of the MA interaction over time (Periods). Six trend components of the PMA are significant, three of them at $p < .005$, two at $p < .01$ and one at $p < .05$. Together they represent 32% of the total PMA variation. The complexity of components, ranging from the first to the fourth powers suggests highly complex surfaces, as depicted in the Figures. It should be observed that when the alcohol dose equals zero the saw-toothed meprobamate effect begins to emerge at period 6 and continues through period 13. Maximum effects generally appear at the higher doses of both drugs. There are large depressions in the surfaces, primarily at .25 and .50 g/kg alcohol. From these Figures it is difficult to characterize the nature of the joint action for two reasons: (1) the surfaces are extremely complicated, and (2), we know that administered dose (oral in this case) of meprobamate is not a good representation of circulating levels (BMC), and this may be true for BAC also.

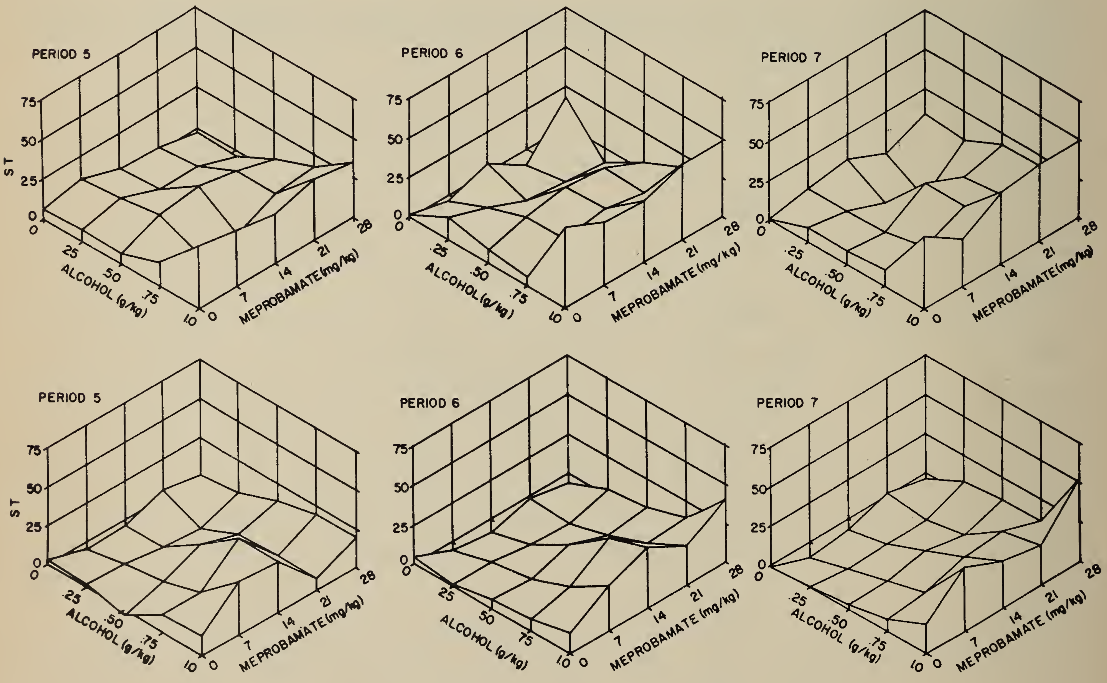

Figure 10. *ST* as a Function of the Doses of Alcohol and Meprobamate in Experiments 4 and 5 for Periods 5, 6 and 7. Upper Curves Are for Experiment 5. *See text for explanation.*

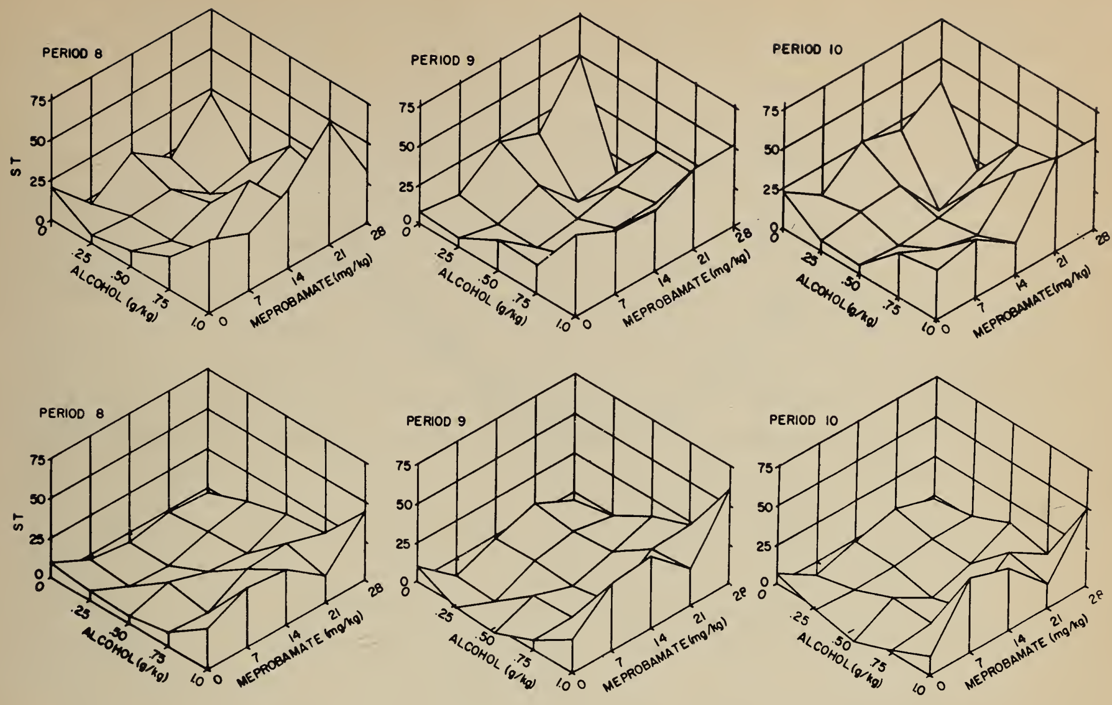

Figure 11. *ST as a Function of the Doses of Alcohol and Meprobamate in Experiments 4 and 5 for Periods 8, 9 and 10. Upper Curves Are for Experiment 5.*

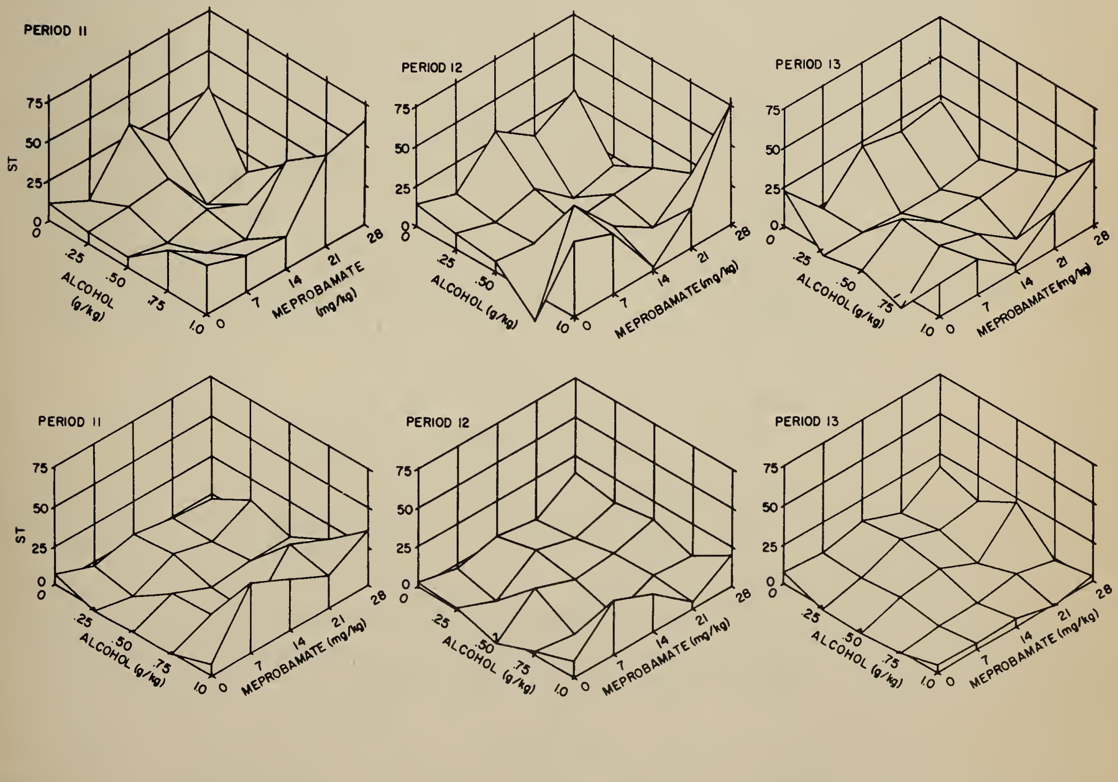

Figure 12. *ST as a Function of the Doses of Alcohol and Meprobamate in Experiments 4 and 5 for Periods 11, 12 and 13. Upper Curves are for Experiment 5.*

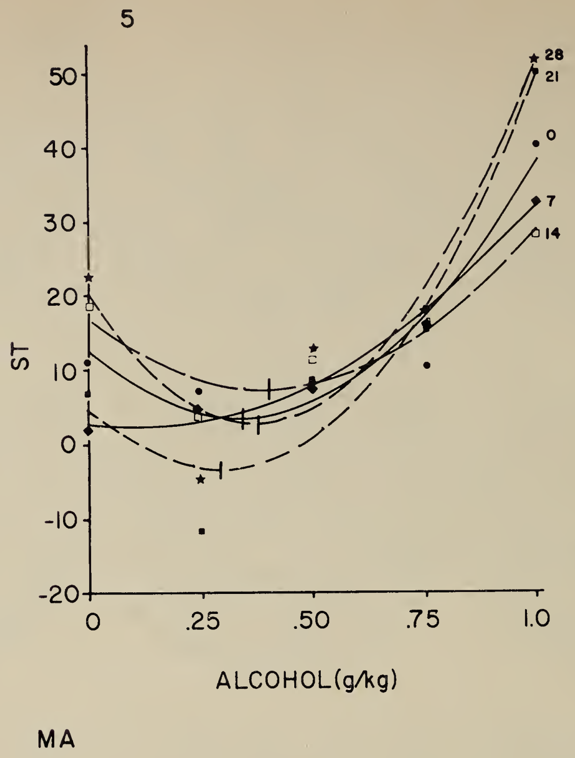

Figure 13. *ST as a Function of Alcohol for Each Dose of Meprobamate (Experiment 5).*

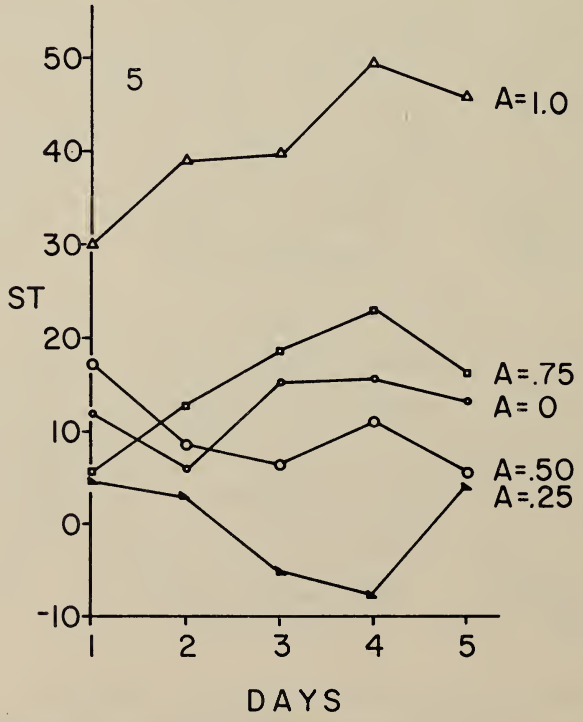

Figure 14. *ST as a Function of Days for Each Dose of Alcohol (Experiment 5).*

Another point that can be made is that alcohol does not seem to be functioning as it did in Experiment 3 as visible from the zero meprobamate curves of Figures 10, 11 and 12. The MA interaction shows this, Figure 13. With the exception of the 7 mg/kg meprobamate curve alcohol produced minima (the verticle lines in Figure 13) between the .25 and .50 g/kg doses. The overall minimum is calculated to be A = .32 g/kg and is significantly different from the zero dose of alcohol (p = .025, one-tail). Some light is thrown on this by examining the change in the effect of alcohol over experimental days. Figure 14 shows the Day X Alcohol interaction. Because Days and Meprobamate are two of the orthogonal Latin square subplot variables in Experiment 5, no interaction between them can be calculated. This means that the Day variable occurs at an average value of meprobamate equal to 14 mg/kg. With this in mind the curves in Figure 14 are somewhat surprising. The important characteristic is that the separate doses do not have the same effect from day to day. In particular, the A = .25 g/kg curve decreases to an apparent minimum at day 4, then increases. The A = .50 g/kg curve decreases over its whole length and the A = .75 and 1.0 g/kg curves increases. The phenomenon is sharper for Targets Missed and Errors, where the day 1 results are more similar to the Experiment 3 results. The Days X Alcohol interactions are not significant for any of the measures but components of the triple interaction are (Periods X Day X Alcohol), which suggests that for some point in time the DA interaction would be significant.

No explanation comes to mind for these results. The shape of the curves seen in Figure 14 for the single curves of the DA interaction have been seen before. Carpenter *et al.* and Carpenter and Ross report the same shape curves with minima in the same region of the alcohol dose.[17, 18] In these latter experiments data were gathered for four days with at least one rest day between experimental days. Carpenter and Ross suggest that different initial levels of skill of the subjects are responsible for the curves. The very best subjects had linear curves of impairment while the poorer subjects had curves similar to the results shown here. In the Carpenter *et al.* and Carpenter and Ross experiments it is impossible to evaluate dose over days because of the Latin square designs. These phenomena require further experimental examination.

In Experiment 4 (chronic administration of meprobamate), performance was superior overall to that of Experiment 5 and the performance curves for the two drugs are frequently quite different from their analogs of Experiment 5.

DISCUSSION

The models require a description of each drug acting alone over a range of doses. The outcome of any combination of the drugs depends on the degree of overlap in terms of "sites of action." Sites of action does not imply physical location nor is it restricted to a single physiological system. Sites of action could mean an enzyme for which the drug is a substrate, and so on. To describe a monotonic function of dose, a single site of action is required; a nonmonotonic relation requires at least two sites. If a one-site-drug is combined with a two-site-drug the resulting effects will depend on whether the single site of the one-site-drug coincides with either or neither of the sites of the two-site-drug. Therefore there are only three possible outcomes of the combination. Since the behavior of the two-site-drug depends on a difference between the activity of the drug at each of the sites, it makes a difference which of these sites is coincident with the single site of the one-site-drug.

Thus, from separate dose-response functions of each drug, we can characterize the action (minimum number of sites) of each. In turn, we can specify the number of possible different kinds of results a combination can produce. Two drugs each with two sites can produce seven results. The choice between the seven possible results is an empirical matter, *i.e.*, testing the drugs in combination. At this point the economic value of the models becomes obvious, because the models indicate what dose combinations to use in order to distinguish between the underlying coincidence of sites of action. For example, knowing the number of sites of action from the individual dose-response functions, it is conceivable that a single dose of each drug tested in combination will answer this question. In addition, it should be possible to decide from the separate dose-response functions and the models the location of any possible dangerous combinations.

This presentation of the models is supposed to be heuristic. It is highly oversimplified. Although these last remarks would apply in a general way to most mathematical models of joint action, I had those of Ashford and Cobby in mind. Mathematically speaking, their models are simple. No flights into hyperspace or such esoterica are involved in their explication. The models are based on respectable concepts from pharmacology: "mass action," "receptor," "site of action," "physiological system," "association constant," and "intrinsic activity," all of which are built into the models. Conceptually the models cover the same areas that have been dealt with by other model makers and on paper look as if they explain the same kinds of problems, and they suggest results so far not seen. The difficulty is in deciding their validity, for which data are absolutely required.

The question now is to what extent do the results gathered by us become useful in testing some part of the Ashford-Cobby models. Ashford and Cobby[10] prepared curves from Experiment 5 with response as a function of the blood levels of each of the two drugs. The curve for alcohol is nonmonotonic with early improvement in performance (maximum at 10 mg/100 ml BAC), followed by decreasing performance at higher BACs. Meprobamate (BMC) has a shallow curve of decreasing performance over its full length. Both curves are at zero blood levels of the other drug.

The alcohol curve requires a two-site model, the meprobamate, a single site. This means that there are three possible results for the combination depending on the coincidence of the meprobamate site with the two alcohol sites. Unfortunately, because of the choice of doses, and the resulting blood levels of the two drugs, it is impossible to distinguish between the three possible outcomes. The empirical results are fitted equally well by all three models. The strong implication of this is that classical designs, used without prior examination of the action of the single drugs with respect to the models will frequently lead to the same impasse. The models should be used to determine what dose of each drug must be tested in combination in order to distinguish the nature of the interaction of the drugs. Such a procedure should lead to simpler and more quickly conducted experiments.

In order to test the models, higher doses of meprobamate and alcohol are indicated. It is unlikely that a dose of alcohol higher than 1.0 g/kg can be used as it was in these experiments. One of the problems in Experiment 1 was that originally we used 1.2 g/kg alcohol and found that just half of our older, experienced drinkers were unable to manage it. Our experience with meprobamate indicates that we can safely increase the dose, although we gave what appeared to be "maximum" dose, 28 mg/kg (2100 mg for 165-lb man).

Dosage is not a good representation of the quantity of a drug available for action when the drug is administered orally. This is especially true when meprobamate is administered in combination with alcohol, and possibly in other drug-alcohol combinations. The relationships of behavior to a range of doses of a drug need not be linear or monotonic. Conclusions based on just two doses (or blood levels) from any non-linear curve will be incorrect as to the action of single drugs and their combination. The shapes of the curves and time of testing must be known rather than assuming that they are linear. Even if definitions of terms expressing the outcome of drug combinations (e.g., additive) were agreed upon, it would be impossible to reach a conclusion by visual examination of the results of the combinations of the two drugs used here. Some other more critical procedure must be used. This is supplied by mathematical models of joint action, which include operations for deciding on agreement between definitions and empirical results. If the present models are found to be valid, the procedure for determining the outcome of a combination is conceptually simple: first, the dose-response functions for each of the drugs alone are determined. Second, from these functions, two sets of models are selected. In the initial set, the models are fitted to each drug acting alone. Then, based on the single-action models, a second set of models can be fitted for the drugs acting together (the number of joint-action models will depend on the character of the single-action models). Third, a choice must be made among the resulting joint-action models. This is accomplished by choosing dose combinations of the two drugs in a region of the joint-action models for which the curves are different. These dose cominations are then tested empirically. The results determine which is the proper model of joint action for the two drugs used. The same procedure can be used for any number of drugs.

ACKNOWLEDGEMENTS

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Action and Interaction of Psychoactive Drugs on Nerve Impulse Conduction and Release of Neurotransmitters

P. Seeman, M.D., Ph.D.

In understanding the actions and interactions of drugs used in clinical practice as well as in non-medical drug usage, it is essential to consider the fundamental effects that psychoactive drugs have on excitable membranes. Combinations of psychoactive drugs can have either synergistic, antagonistic, or simply additive effects; and such effects can often be explained by a fundamental consideration of events occurring on single axons or at a single synapse.

There are at least 5 sites in a synapse that are highly vulnerable to modulation by drugs. As shown in Figure 1, these are: (1) invasion of nerve impulses into the preterminal axon; (2) stimulus-response coupling; *i.e.*, blockade of the deplorization-secretion coupling, and/or blockade of depolarization-induced entry of Ca^{++};[1] (3) release of neurotransmitter; (4) re-uptake of transmitter or its precursor; (5) post-synaptic receptor region.

Although most psychoactive drugs can affect all these 5 sites to varying degrees, there is not a single psychoactive drug for which we have dose-response data for the action of the drug on all these 5 sites. A thorough understanding of the actions of a psychoactive drug would in fact require such information. The discussion in this manuscript deals primarily with sites 1 and 3.

IMPULSE BLOCKADE BY PSYCHOACTIVE DRUGS

The majority, if not all, of the lipid-soluble psychoactive drugs can block the conduction of nerve impulses.[2] Impulse-blockade can occur at rather low concentrations, such as in the micro-molar and nano-molar concentration regions for neuro-

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leptics. The concentrations which produce 50% blockade of the compound action potential of rat phrenic nerve fibers[3,4] are shown in Table I.

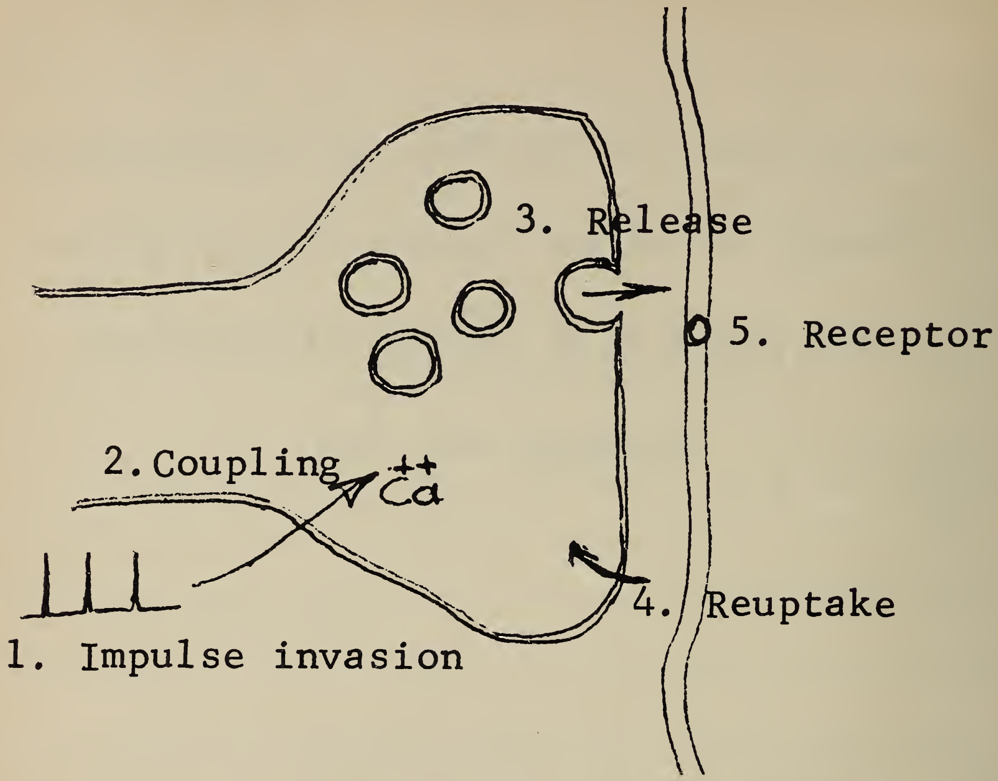

Figure 1. *Depicting the sites most vulnerable to blockade by psychoactive drugs.*

TABLE I

BLOCKADE OF ACTION POTENTIAL

Anti-convulsants:	
diphenylhydantoin	2.7×10^{-5} M
Sedatives:	
phenobarbital	8.0×10^{-4} M
pentobarbital	3.7×10^{-4} M
Neuroleptics:	
chlorpromazine	3.5×10^{-7} M
haloperidol	1.2×10^{-7} M
trifluperidol	5.1×10^{-8} M
thioridazine	1.6×10^{-7} M
moperone	7.6×10^{-8} M
Alcohols:	
ethanol	4.4×10^{-1} M
benzyl alcohol	4.0×10^{-3} M
Opiates:	
methadone	5.0×10^{-6} M
levorphanol	2.0×10^{-4} M
dextrorphan	3.5×10^{-4} M
naloxone	4.0×10^{-4} M
Anti-depressants:	
imipramine	2.6×10^{-6} M
Anesthetics:	
urethane	1.1×10^{-1} M
procaine	2.5×10^{-4} M
lidocaine	1.0×10^{-4} M
procainamide	1.2×10^{-2} M

Drug concentrations even lower than those in Table I would be expected to block nerve impulse conduction in the central nervous system, since it has been found that small diameter neurones are much more susceptible to blockade.[4]

FUNDAMENTAL MEMBRANE ACTIONS OF PSYCHOACTIVE DRUGS

Of the many fundamental actions of psychoactive drugs on membranes,[2] there are 3 of outstanding importance. These are (a) Membrane expansion, (b) Membrane fluidization, and (c) Effects on membrane calcium.

Membrane expansion: An example of membrane expansion by chlorpromazine is shown in Figure 2, where a high precision density meter was used to measure the specific density, in g/ml, (or the specific membrane volume, in ml/g) of synaptosome membranes.

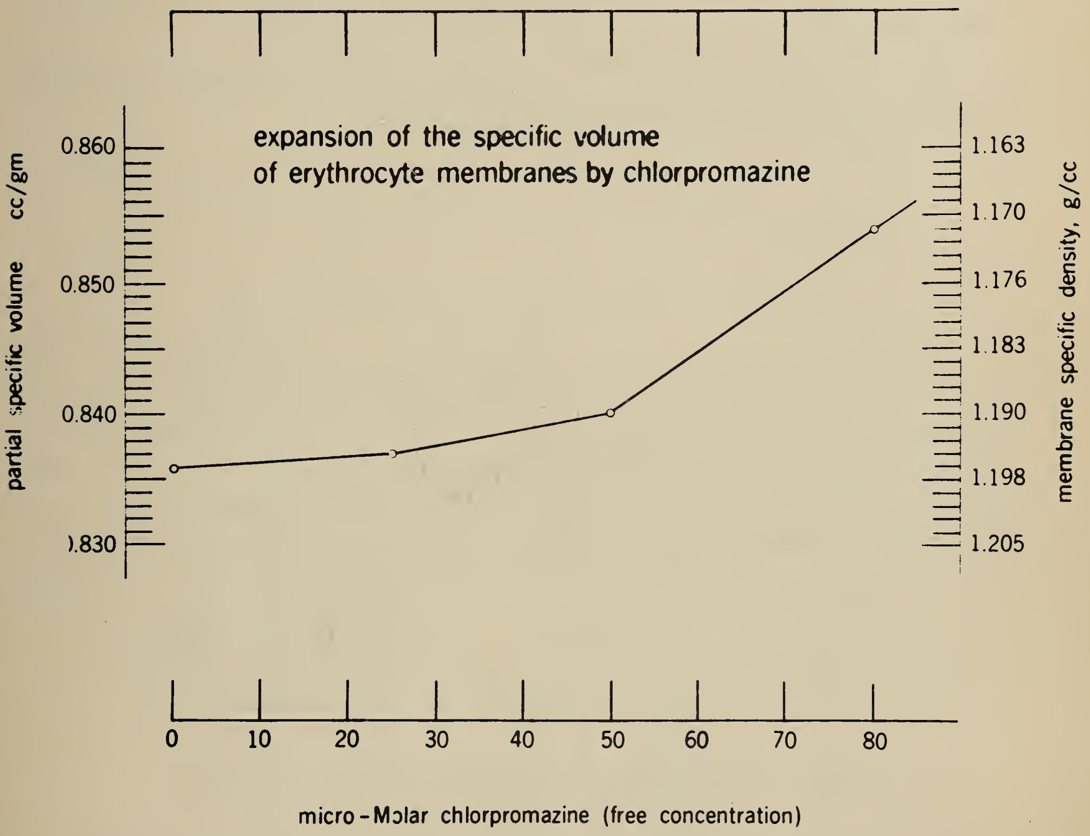

Figure 2. *Membrane expansion by chlorpromazine. Synaptosome membranes were also expanded by chlorpromazine. 25°C.*

All the lipid-soluble nerve-blocking drugs are effective in expanding membranes. In the case of alcohols and anesthetics, the membrane expansion is about 0.5% under conditions of general anesthesia. It is both important and interesting that recompression of animals anesthetized with alcohol or general anesthetics can suddenly reawaken the animals even though the drug is still present.[5]

The general evidence available indicates that membrane expansion results in a distortion of the Na^+-conductance channel responsible for the nerve impulse;[2] that is, membrane expansion produces *electrical stabilization* of the membrane. The expansion occurs primarily in the proteins of the membrane since separate experiments have shown that pure lipid membranes expand by only 0.01% at concentrations which expand the biological membranes by 0.5%. Membrane expansion would also affect, therefore, membrane receptors as well as membrane enzymes.

Membrane fluidization: At the same time as the membrane is expanded by the lipid-soluble drug, the membrane viscosity falls, and the membrane is "fluidized".[6] This fluidization may be the underlying explanation of the ethanol-induced increase in neurosecretion at cholinergic synapses.[1, 2, 7-9]

Drug effects on membrane-bound calcium ions: The psychoactive drugs which are tertiary amines adsorb to the membrane in very high concentrations, and thus displace the membrane-bound Ca^{++}.[2] The neutrally charged psychoactive drugs, however, either have no effect on the membrane-bound Ca^{++} or cause it to rise.

Drug interactions at the level of the axon (Nerve impulses): On the limited basis of only one study,[10] it appears that neutral anesthetics (benzyl alcohol) and tertiary amine anesthetics (procaine) are simply additive in blocking nerve impulse conduction; no antagonism or synergism was observed.

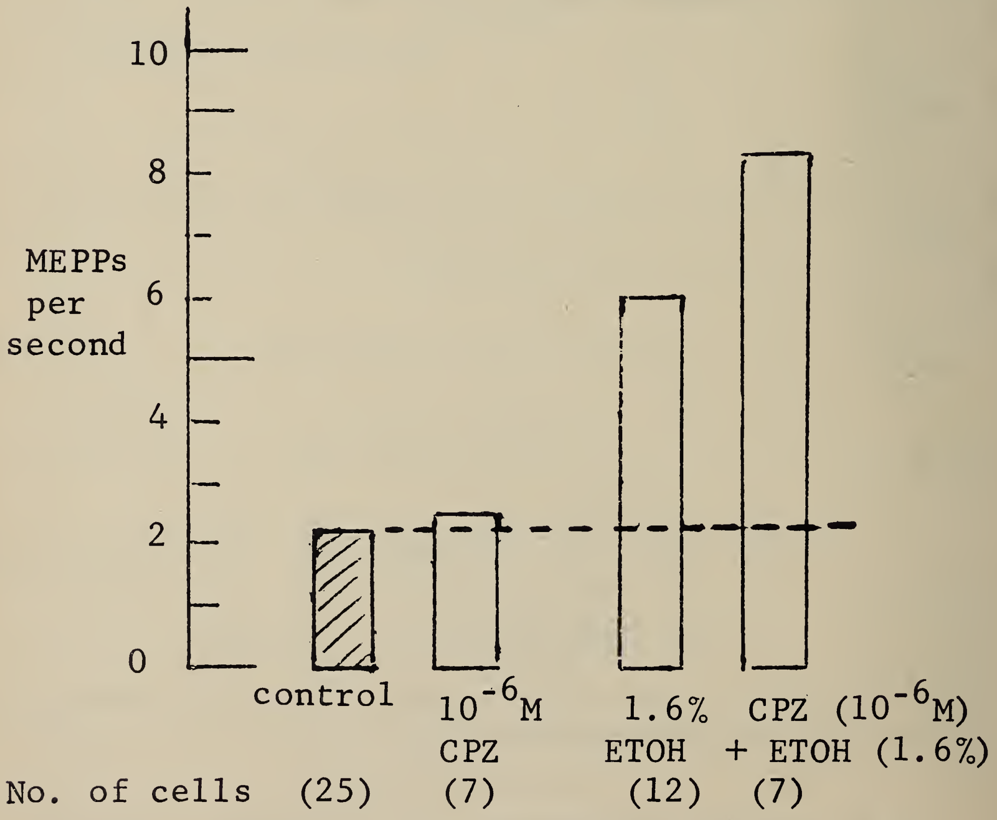

Figure 3. *Showing that* 10^{-6}*M chlorpromazine, while having only a small effect of its own, potentiates the action of ethanol in increasing the frequency of miniature end-plate potentials (MEPPs) in the rat phrenic nerve-diaphragm.*

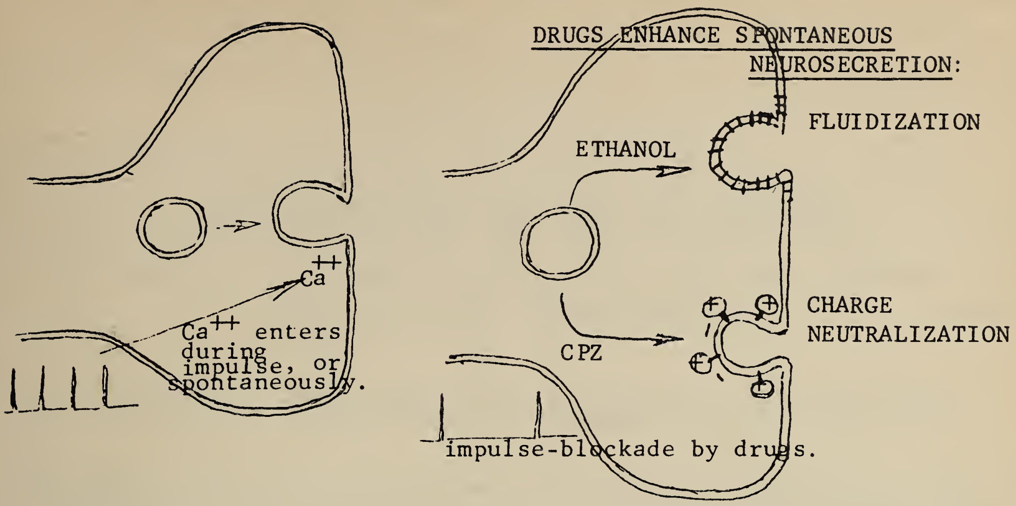
Ca^{++}
Ca^{++} enters during impulse, or spontaneously.
DRUGS ENHANCE SPONTANEOUS NEUROSECRETION:
FLUIDIZATION
ETHANOL
CHARGE NEUTRALIZATION
CPZ
impulse-blockade by drugs.

Figure 4. *Depicting the hypotheses that ethanol increases MEPP frequency by fluidizing both the vesicle membrane and the pre-synaptic membrane to result in fusion, while chlorpromazine enhances MEPP frequency by neutralizing the surface – negative charge of the membranes. A combination of ethanol and chlorpromazine (Fig. 3), therefore, would be expected to result in synergism.*

DRUG INTERACTIONS AT THE LEVEL OF THE SYNAPSE

There is no published information available on drug interactions at single snyapses. The data in Figure 3 show an example of work currently being done in this laboratory. It demonstrates that although 10^{-6}M chlorpromazine barely increased the MEPP frequency (miniature end-plate potential frequency), this concentration potentiated the action of ethanol. The hypotheses shown in Figure 4 explain that such potentiation is understandable considering the fact that ethanol promotes release without needing Ca^{++}[8] while chlorpromazine promotes release by displacing membrane-bound Ca^{++}.

It appears, therefore, that the well-known susceptibility to intoxication in persons taking neuroleptics has its corollary in the simple nerve-muscle junction.

DRUG INTERACTIONS IN THE WHOLE NERVOUS SYSTEM

As pointed out by Esplin,[11] it is inappropriate to emphasize the clinical specificity of drugs acting in the central nervous system because there is much overlap between effects which are termed sedative, tranquillizing, anti-depressant, pain-relieving, anesthetic, anti-convulsant, etc. It can be shown, for example, that convulsant fluorinated compounds also have an anesthetic component, while some general anesthetics may themselves have an anesthetic-antagonizing component. These contrary properties can be brought out by testing combinations of anesthetics[12, 13] (isobolographic pharmacology).

Presumably, the excitement-producing aspects of these anesthetics (e.g. cyclopropane) may be based either on the blockade of inhibitory cells, or enhanced neurosecretion (fluidization) from excitatory neurones. The physico-chemical differences between flurothyl (a convulsant) and isoflurothyl (a general anesthetic), a structural isomer of flurothyl, are small, yet the two molecules result in radically different clinical actions on the entire nervous system.[2]

In summary, simple additive effects may occur between lipidsoluble psychoactive drugs if they act on nerve impulses, while potentiation may occur if the two drugs act on the same type of synapse, with antagonism occurring if the drugs act at different synapses or different receptors. *A priori* predictions of the final effect of a drug combination, therefore, are virtually impossible with our present state of knowledge.

ACKNOWLEDGEMENT

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Mechanism of the Synaptic Effects of Morphine, Indomethacin and Prostaglandins

Seymour Ehrenpreis, Ph.D. and Joel Greenberg, B.S.

ABSTRACT Studies were undertaken on the electrically stimulated guinea pig ileum to attempt to understand the mechanism by which various minor and potent analgesics affect synaptic transmission. In this way a better understanding of such effects as analgesia, tolerance and withdrawal could be obtained. Morphine blocks transmission by inhibiting release of acetylcholine; naloxone antagonizes the block by competing for the morphine receptor ("T" receptor). Naloxone also causes a contracture of the tissue when added after morphine. This contracture results from displacement of morphine from a second receptor ("I" receptor) which may be on the synaptic vesicle. This effect may account for some of the symptoms observed during precipitated withdrawal. Evidence is presented to implicate the "I" receptor in tolerance; the "T" receptor may correspond with the CNS receptor responsible for analgesia. Prostaglandins (PGs) of the E series selectively reverse block of transmission by morphine and other opiates and it is suggested that the "T" receptor is actually a prostaglandin receptor. It is proposed that PG acts as a modulator of acetylcholine transmission in the ileum. This was confirmed by the finding that indomethacin, an inhibitor of PG synthesis, blocks transmission; this effect is reversed by very low concentrations of PGE_1 or E_2. This occurs whether the tissue is exposed to indomethacin *in vitro* or if the drug is injected. Thus it is proposed that the central effects of morphine and other analgesics are produced by the selective inhibition of cholinergic transmission. These drugs have little if any effect on adrenergic transmission, for example in the vas deferens.

Studies by Paton,[1] Schaumann[2] and co-workers have shown that morphine and other opiates block transmission in the guinea pig ileum by inhibiting the release of acetylcholine (ACh) from the nerve network in the tissue (Auerbach's plexus). This effect comes about through the combination of these drugs with a receptor present in the cholinergic nerve terminals. Based on the close correspondence between affinity of a large series of drugs for this receptor and their analgesic potency[3] it may be concluded that the receptor in the myenteric plexus of the ileum is very similar to the analgesic receptor in the CNS. Indeed, in a recent study Pert and Snyder[4] demonstrated the same type of stereospecific binding of naloxone, the opiate antagonist, to the ileum as well as the CNS.

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The purpose of this communication is several fold: to present evidence that more than one opiate receptor is present in the guinea pig ileum; to indicate how these receptors may be involved in tolerance and withdrawal; to suggest the localization of these receptors in the nerve network of the tissue; and to demonstrate that one of these receptors is actually a receptor for prostaglandin which plays an essential role in controlling ACh release and hence transmission events in this tissue.

THE ELECTRICALLY STIMULATED GUINEA PIG ILEUM

Following excision of the ileum, the tissue is thoroughly cleaned of contents and cut into pieces 2-3 cm long. The tissue is then mounted in the organ bath as described by Paton and co-workers[1] and stimulated at a frequency of 0.2Hz (1 shock/5 sec.), duration 0.4-0.8 msec, 40 volts. Tyrode's solution is used, the bath temperature is 37C, and a mixture of 95% oxygen 5% CO_2 is bubbled through the solution.

Evidence for Two Opiate Receptors

When morphine is added to the bath a progressive block of contractions is observed. Complete block occurs at approximately 50ng/ml. As shown in Figure 1 naloxone very effectively reverses the block. In Figure 2 naloxone at a fairly high concentration (16ng/ml) is added and, as may be noted, reversal is accompanied by a marked contracture of the tissue. It was possible to effectively dissociate the contracture produced by naloxone following morphine from the effect on morphine block of contractions in several ways: 1. If the time interval between addition of naloxone following morphine was less than about 10 min. then only reversal of the block was observed (Fig. 3). 2. Morphine block could be reversed simply by washing the tissue with buffer (Fig. 4). If at this time naloxone is added, a marked contracture is noted. In other words, although complete transmission had been restored, contracture was still possible, suggesting that morphine remained in the tissue but not in a form influencing transmission. It should be noted that naloxone has no effect on electrically-induced contractions of a tissue not previously exposed to morphine. 3. If naloxone is added to the ileum from an addicted guinea pig (100 mg/kg morphine, 10 days) a marked contracture is observed (Fig. 5) with little if any indication of improvement in electrically-induced contractions. Thus once again, considerable morphine remains within the tissue but in a state which does not influence transmission.

It can be shown that the naloxone-induced contracture results from the outpouring of ACh from the tissue. Thus the contracture is readily and rapidly reversed in the presence of low concentrations of atropine (Fig. 6).

These findings led to the suggestion[3, 5] that two receptors for morphine exist in the tissue: a "T" receptor, located on the nerve terminal membrane, involved in the control of ACh release, and an "I" receptor located more internally in the terminal. The "I" receptor may be present on the synaptic vesicle. When morphine is displaced from this site by naloxone, the vesicle ruptures and liberates its content of ACh. Direct examination of morphine-naloxone interaction with synaptic vesicles from the brain confirmed this hypothesis (D. Teller, personal communication).

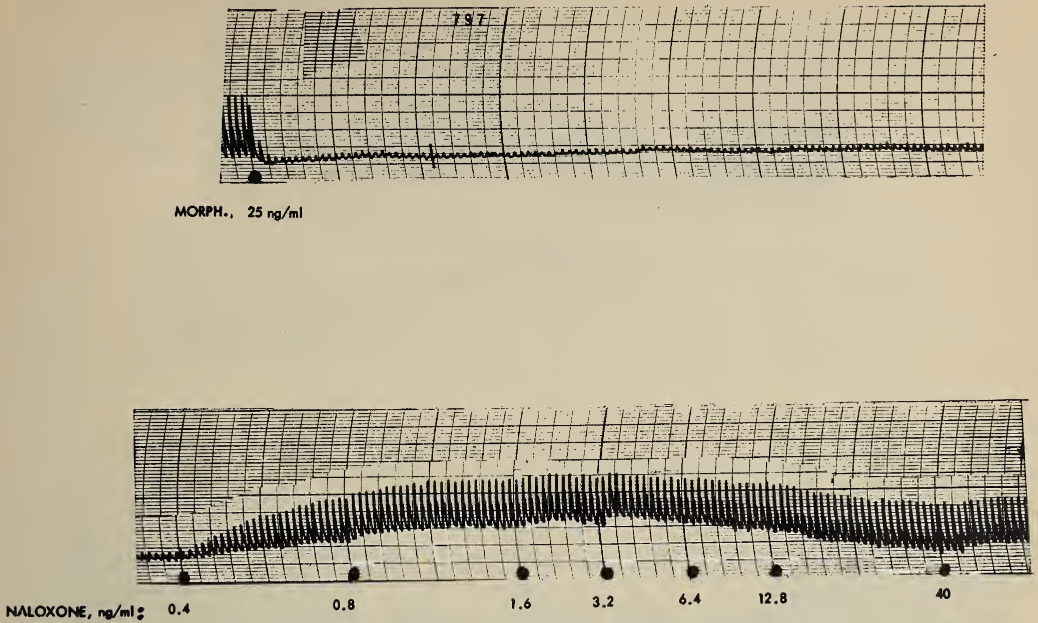

Figure 1. *Morphine-naloxone antagonism on electrically stimulated guinea pig ileum. The concentrations of the various drugs are shown on the figure.*

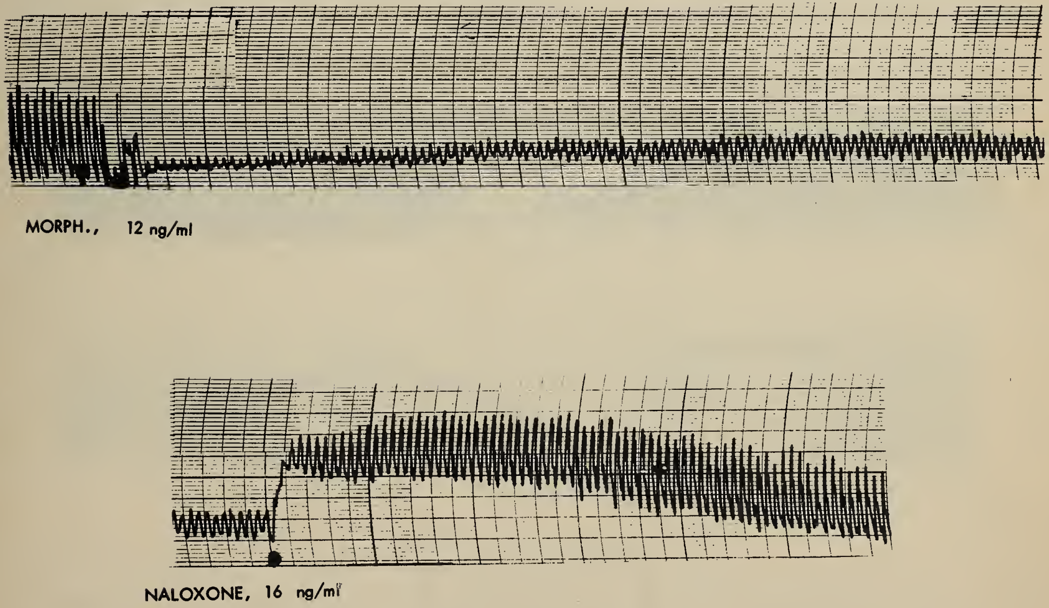

Figure 2. *Naloxone-induced contracture of the ileum following exposure to morphine.*

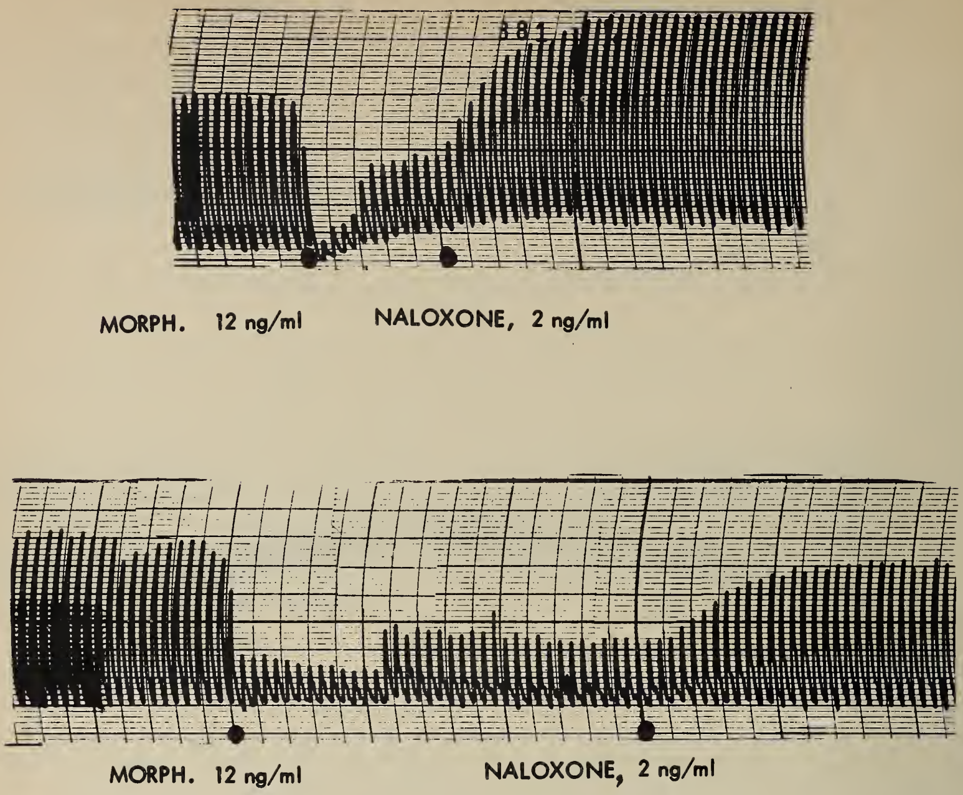

Figure 3. *Time dependence of naloxone-induced contracture following morphine. Upper trace: Naloxone added 1 minute after morphine. Lower trace: Naloxone added 3 minutes after morphine. Note only hint of rise in base line tension under these conditions of short exposure to morphine.*

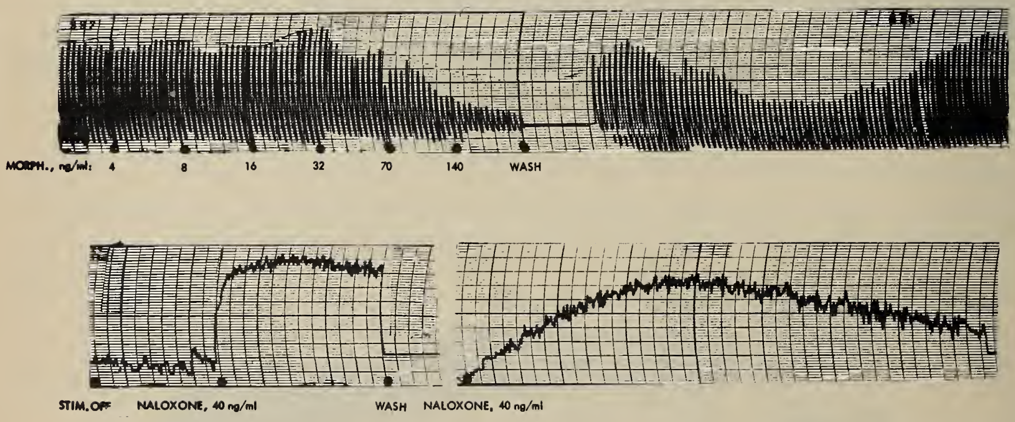

Figure 4. *Dissociation of naloxone contracture from improvement of contraction following morphine exposure. Morphine was first titrated in to complete block and the tissue was washed for 1 minute. Following recovery of transmission, addition of naloxone results in a marked contracture. A second large contracture is noted following washout of the first dose of naloxone.*

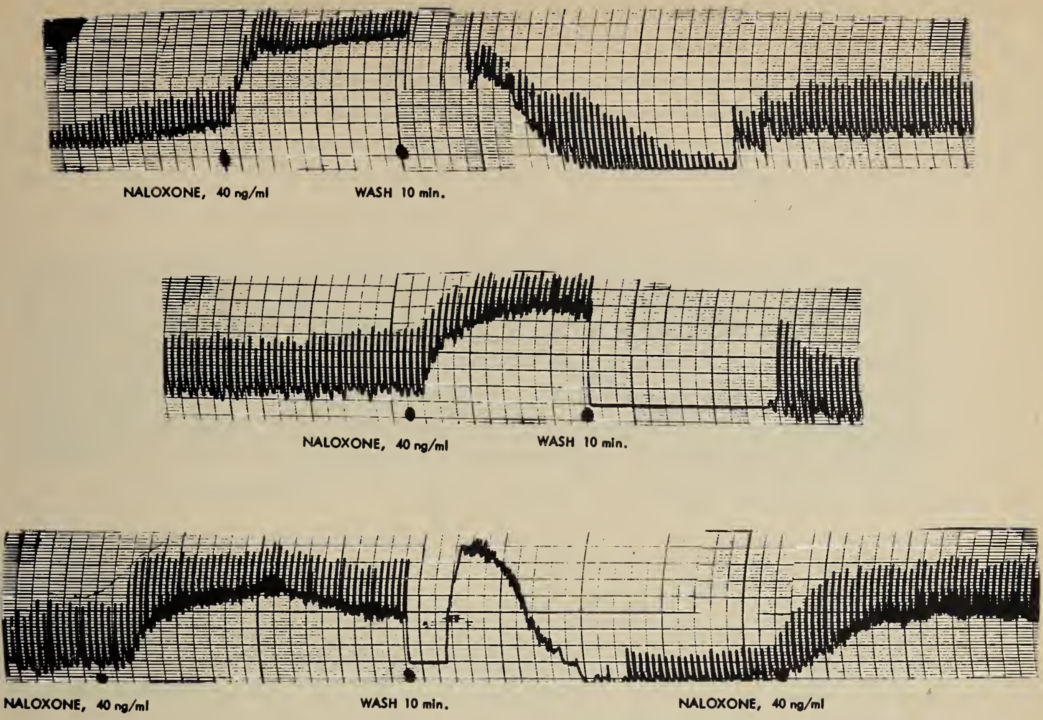

Figure 5. *Naloxone contracture of ileum removed from addicted guinea pig. Note that large contractures can be elicited following several additions of naloxone interspersed by wash of the tissue.*

Involvement of the "I" Receptors in Tolerance

A control and addicted guinea pig were injected with a very large dose of morphine (500 mg/kg); two hours later the ilea were subjected to electrical stimulation. It is apparent that whereas in the control ileum transmission was completely blocked, the addicted ileum was normal (Figure 7). Naloxone had the expected actions on these two ilea: a marked contracture in the addicted ileum, restoration of transmission in the control. Since the "T" receptors of the addicted ileum more or less have the usual affinity for morphine, it is evident that the resistance shown to the injected morphine was due to some profound change in the "I" receptors. It has been suggested that during tolerance this receptor increases in number or affinity for morphine[3] and that the drug is bound so strongly to this site that the concentration in the presence of the "T" receptor fails to reach that required to block transmission.

Involvement of the "T" and "I" Receptors in Withdrawal

During withdrawal, particularly when precipitated by a narcotic antagonist, the following occurs: large amounts of ACh are liberated from the synaptic vesicles due to displacement of the narcotic. The liberated ACh interacts with its own postsynaptic receptors thereby producing symptoms of cholinergic stimulation. Thus withdrawal symptoms are possible in the face of normal transmission since, as discussed above, morphine bound to the "I" receptors does not influence transmission. The morphine is very tightly bound to this site and thus withdrawal symptoms may be elicited several days following cessation of administration of the opiate. Binding studies with radioactive morphine clearly show very strong interaction with some internal site in the ileum.[6]

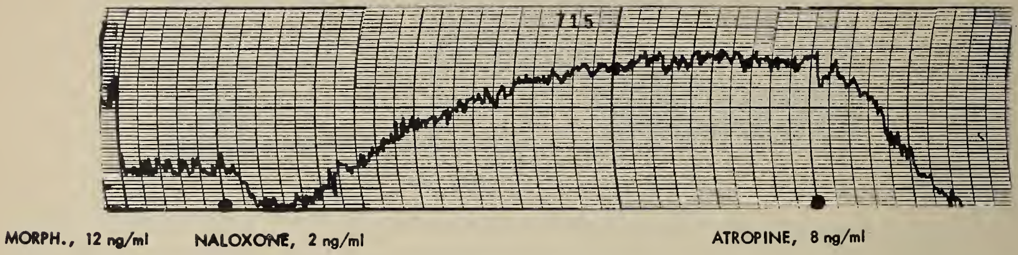

Figure 6. *Evidence that naloxone-induced contracture of ileum following morphine is mediated by acetylcholine released from the tissue. Morphine, 12ng/ml was added for 10 minutes following which the stimulator was shut off and naloxone added. A very low concentration of atropine, added at the height of the contracture, causes a rapid reversal of the contracture.*

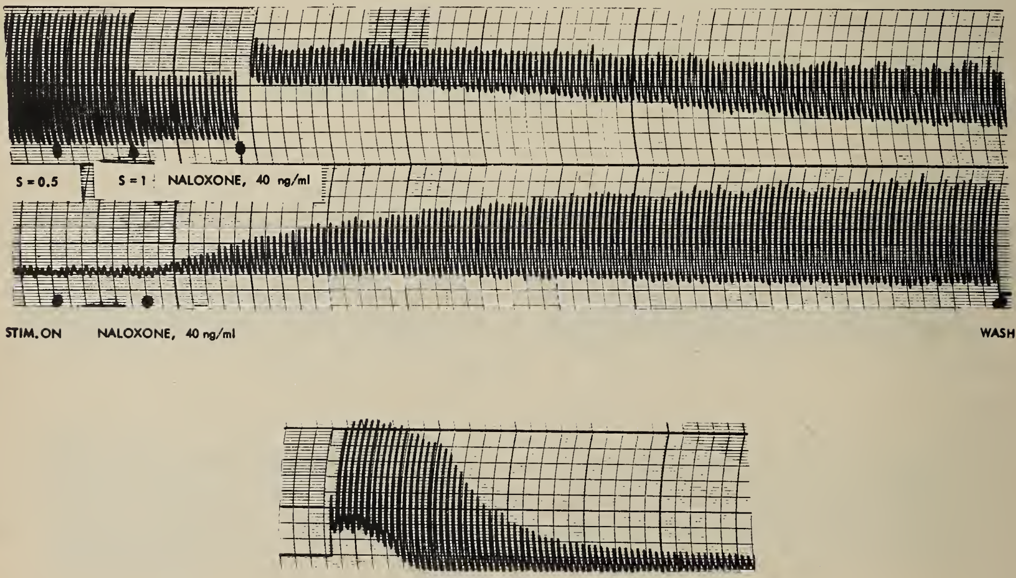

Figure 7. *Effect of injecting a very large dose of morphine on electrically induced contractions of the ileum. Upper tracing: Ileum from addicted animal. Naloxone causes a large contracture without improvement in contraction height. Lower tracing: Ileum from normal ileum. Note complete block of contractions caused by the injected morphine. Naloxone produces an immediate improvement in contractions but no contracture. Following wash note that contractions gradually fail, indicating the presence of large amounts of morphine within the tissue.*

Evidence that the "T" Receptor may be a Receptor for Prostaglandin

We have discovered that of the many naturally occurring substances tested for ability to reverse morphine block of transmission, only prostaglandins have this property.[7] Figure 8 shows a typical experiment. Figure 9 shows reversal by several PGs, clearly indicating that PGs of the E series are most potent. Competition between PGE_1 and morphine for a common site is indicated by data of Figure 10. In Figure 11 it is shown that PGE_2 fails to reverse block of contractions caused by non-opiates such as phenobarbital or norepinephrine. PGE_2 fails to reverse block of contractions by benadryl and various local anesthetics.

In addition, it was ascertained that a number of smooth muscle stimulants failed to reverse the block by morphine. This was necessary because PGE_1 and E_2 cause a contraction of the tissue. The data of Figure 11 show that smooth muscle stimulants such as serotonin, bradykinin or dimethylphenyl piperizinium (DMPP) fail to significantly reverse morphine block when added at concentrations which produce significant contractions of the tissue.

The preceding results provide strong evidence that the morphine antagonism by PG of the E series is highly specific and most likely of a competitive nature. It is suggested that morphine and other opiates, all of which are antagonized by PGE_1 or E_2, combine with a PG receptor preventing endogenous PG from performing its function of modulating ACh release. Thus the opiates behave as receptor inhibitors or antagonists and PG is the agonist.

In confirmation of this concept of the presence of a PG receptor in the ileum and its role in controlling synaptic transmission, we have found that compounds known to act as PG receptor antagonists on other tissues also block transmission in the ileum. These include 7-oxa-prostynoic acid[8] and SC19220.[9] As predicted, PGE_2 reversed the block produced by these compounds at very low concentrations (several ng/ml).

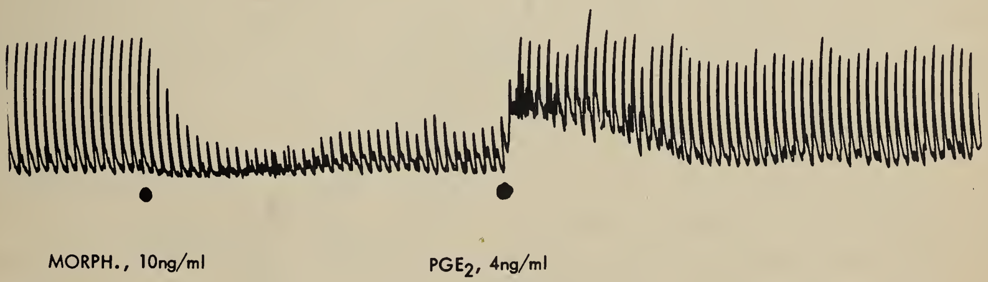

Figure 8. *Reversal of morphine block of contractions of electrically stimulated guinea pig ileum by PGE_2. The morphine, 10 ng/ml, was applied for 5 minutes followed by addition of the prostaglandin. Recovery is approximately 80%.*

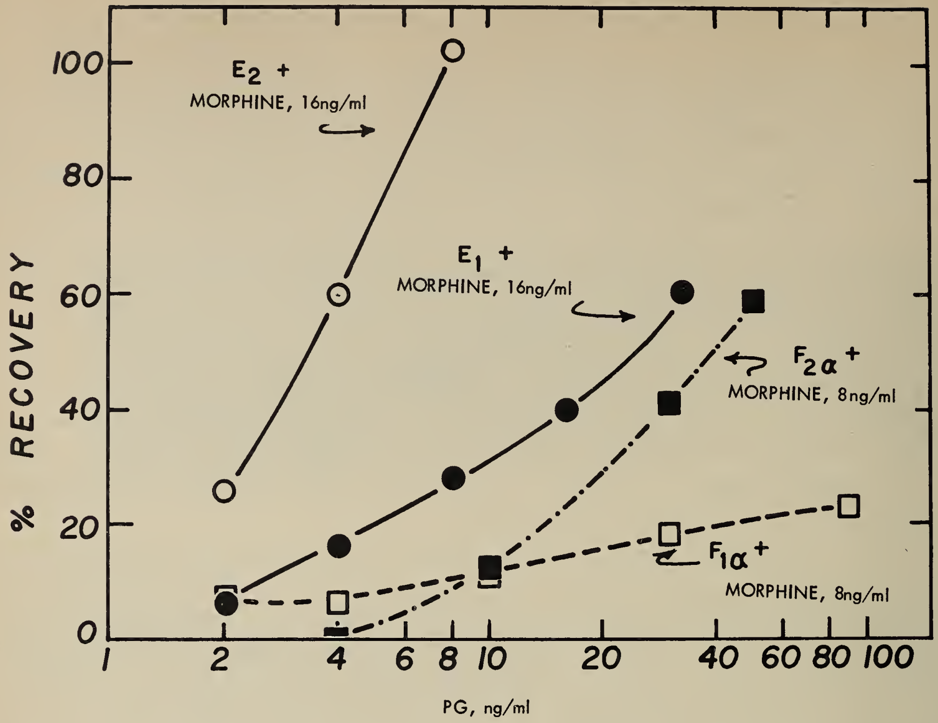

Figure 9. *Cumulative dose-response curves for reversal of morphine block by various prostaglandins. Note that in the case of PGF*$_{1\alpha}$ *and F*$_{2\alpha}$ *the concentration of morphine was half that with PGE*$_1$ *and E*$_2$. *Reversal by F*$_{1\alpha}$ *was essentially absent. In each experiment, the tissue was exposed to the morphine for 5 min. prior to adding the particular PG.*

In vitro Effects of Indomethacin and Aspirin on Transmission in the Guinea Pig Ileum

In order to confirm the essentiality of PG for cholinergic transmission in the ileum the effects of indomethacin and aspirin were studied. It is well known that these drugs are very effective in inhibiting synthesis of PG; once this occurs, the endogenous level of PG should drop fairly rapidly due to metabolism by various degradative enzymes resulting in transmission failure. Figure 12 is a typical experiment showing that transmission is indeed blocked in the presence of indomethacin. Of considerable importance is the fact that transmission is readily restored by very low concentrations of PGE$_1$ or E$_2$. In addition, it may be seen that following wash-out of the PG the block is reinstated and that again PG restores transmission at this point. This finding is of particular interest because it has been shown by Smith and Lands[9] that indomethacin can irreversibly inhibit dioxygenase, the enzyme directly involved in the synthesis of prostaglandin. Irreversible inhibition occurs in the same concentration range of indomethacin as is required for blocking transmission in the ileum. Thus these results strongly suggest the essentiality of the enzyme dioxygenase, as well as PG, in transmission in this tissue.

Table I gives data for the potency of various PGs in restoring transmission in the ileum from which it is apparent that PGs of the E series are by far the most effective.

TABLE I

POTENCY OF VARIOUS PROSTAGLANDINS IN REVERSING INDOMETHACIN BLOCK OF ELECTRICALLY INDUCED CONTRACTIONS OF GUINEA PIG ILEUM

Prostaglandin	ED_{50} for complete reversal,* ng/ml
A_1	340
B_1	70
E_1, E_2	2
F_{2a}	270

*These values refer to reversal of block following washout of the indomethacin

Essentially similar results were obtained with aspirin. However, aspirin proved to be far less effective than indomethacin in blocking transmission. This is in accord with the results of Smith and Lands who showed that the potency of aspirin was almost 100 times less than that of indomethacin in inhibiting dioxygenase.

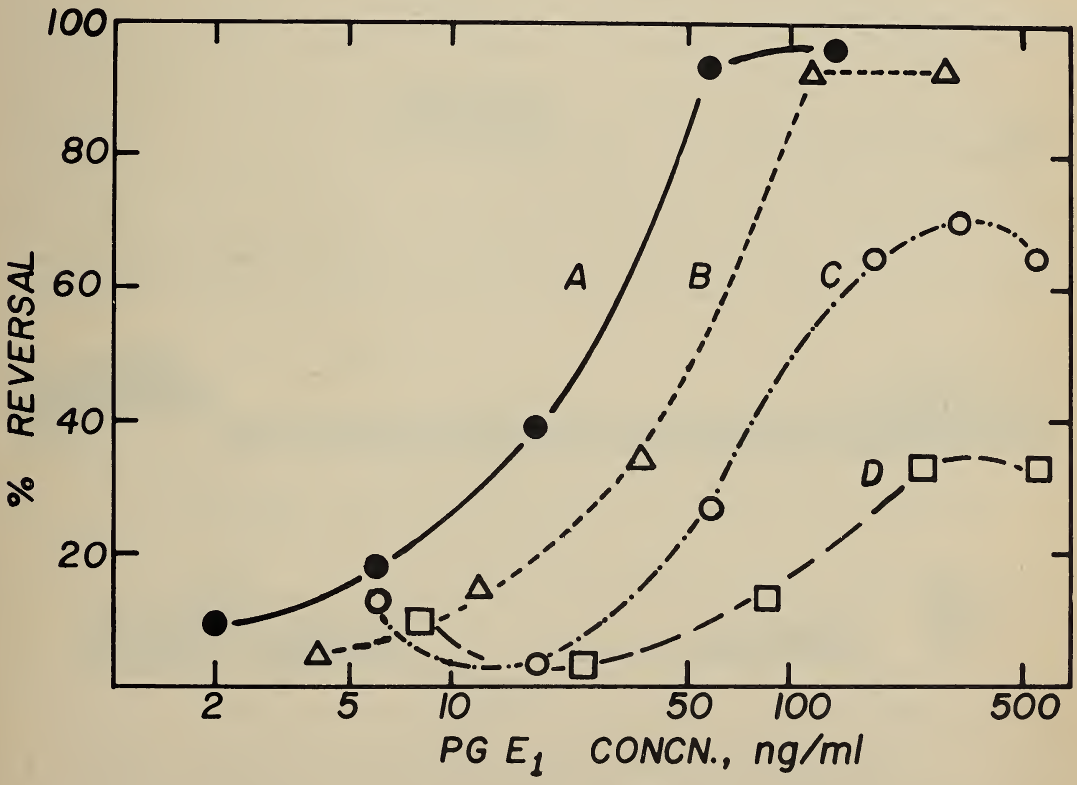

Figure 10. *Cumulative dose-response curves for reversal of morphine block by* PGE_1. *Concentrations of morphine were: A, 20 ng/ml; B, 40 ng/ml; C, 80 ng/ml; D, 160 ng/ml.*

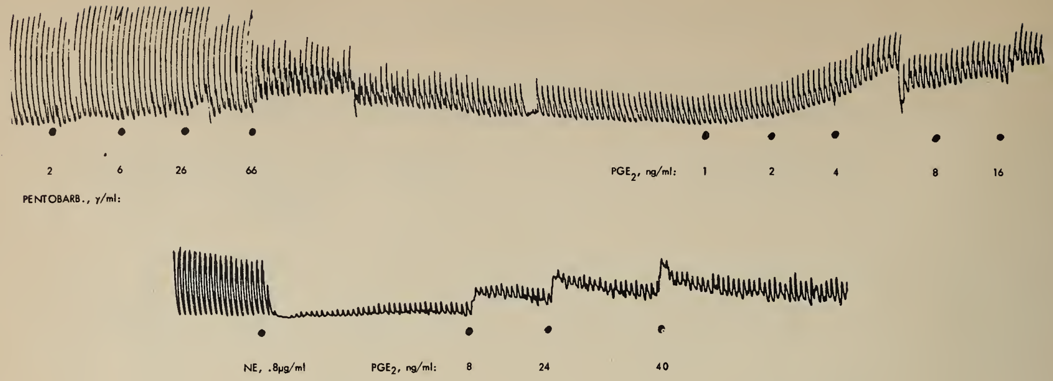

Figure 11. *Failure of PGE_2 to reverse block of contractions of ileum by pentobarbital and norepinephrine.*

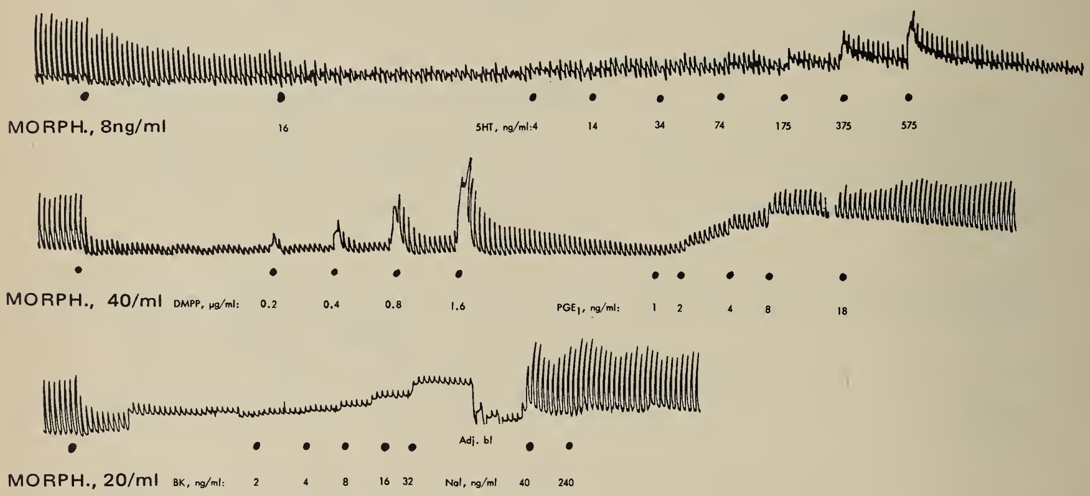

Figure 12. *Failure of smooth muscle stimulants —serotonin, bradykinin and dimethylphenyl piperizinium — to reverse block of contractions of ileum by morphine.*

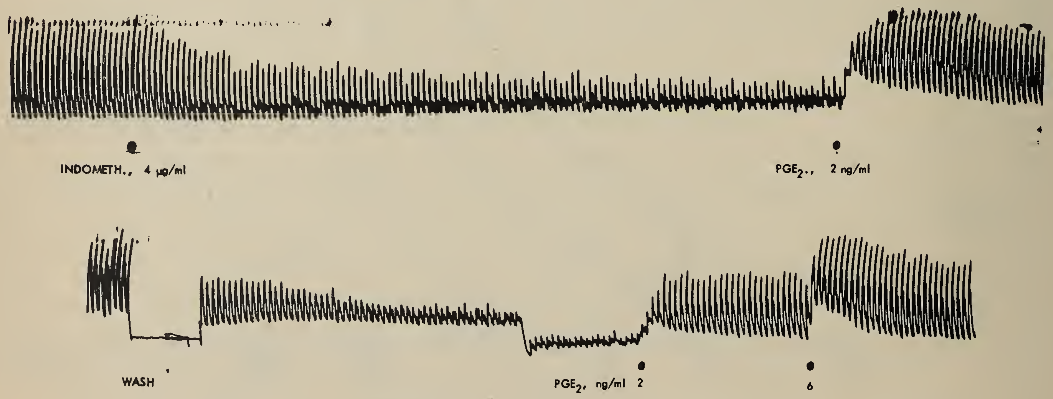

Figure 13. *Inhibition of contractions of electrically stimulated guinea pig ileum by indomethacin, 40μg/ml. Indomethacin remained in the bath for 30 min. Note complete reversal of the block by PGE_2, 2 ng/ml. Following wash, complete block was reinstated after about 15 min. At this point, transmission was restored by very low concentrations of PGE_2.*

Effect of Injected Indomethacin

Injection of indomethacin (30 mg/kg, 3 or 4 times per day) gave results consistent with the *in vitro* studies involving the drug (Figure 13). The ilea from such animals responded very poorly to electrical stimulation; a brief tetanic stimulation (20 shocks/second, 1 minute) resulted in complete block of transmission lasting for exceedingly long periods of time. This too was observed with ilea treated *in vitro* with indomethacin which showed some residual transmission following wash-out of the drug. This residual transmission appears to be mediated by the very small amounts of PG remaining within the tissue, possibly due to incomplete inhibition of synthesis. Tetanic stimulation apparently causes the expulsion of this small amount of PG with subsequent essentially irreversible block of transmission. At this point, addition of very small quantities of PGE_2 (1-2ng/ml) rapidly restores transmission. Thus these *in vivo* results are in complete agreement with those obtained with the drug in the *in vitro* experiments and is further strong evidence for the essentiality of the PG system in cholinergic transmission of the ileum.

Prostaglandins and Adrenergic Transmission

There have been reports of the involvement of a PG system as the modulator for adrenergic transmission, particularly in the vas deferens[11] and the CNS.[12] Contrary to the reports of Hedqvist and co-workers, we have failed to find a significant effect of PGE_2 on transmission in the vas deferens. Furthermore, transmission in this tissue is not affected by indomethacin when added to the isolated tissue or injected into the guinea pig. Thus our evidence indicates lack of involvement of the PG system in transmission in the vas; a similar conclusion was reached by Ambache recently.[13] In accordance with the concept that morphine action on transmission is mediated by a PG receptor, even very high doses of morphine failed to affect transmission in the vas deferens.

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Studies with Methylphenidate: Drug Interactions and Metabolism

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Z. H. Israili, Ph.D., B. A. Faraj, Ph.D., K. Rodewig, M.D.,
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ABSTRACT Methylphenidate was found by us to be metabolized in man by pathways that were predictable—deesterification and hydroxylation at the para-position of the benzene ring. The deesterified compound, ritalinic acid, is a major metabolite, whereas p-hydroxy methylphenidate is a minor one. Bartlett and Egger found another minor metabolite resulting from oxidation at the piperidine ring. Studies of metabolism and distribution suggest that different effects might be expected from intravenous versus oral doses. After intravenous administration plasma levels of unchanged drug are higher than after oral administration. Considerable concentrations of metabolites were found circulating in plasma. This may have implications in the treatment of hyperkinesia (minimal brain dysfunction) for which methylphenidate is the drug of choice. Evidence was presented that, contrary to the report of Kato and Vassanelli methylphenidate is an inhibitor of drug metabolism.

The purpose of this paper is to review the literature concerned with various aspects of the metabolism of methylphenidate. In addition, we wish to present some hitherto unpublished data, including studies of the inhibition of the metabolism of diphenylhydantoin by methylphenidate *in vitro*.

Our interest in the question of metabolism of methylphenidate was aroused by following two patients in whom drug level measurements contributed to a better understanding of their course.

CASE REPORTS

Patient I. We were following this hyperkinetic child for reasons unrelated to methylphenidate. A 5-year-old, 17 kg, Negro boy was observed because of nocturnal grand mal seizures, mental retardation and minimal cerebral dysfunction syndrome.

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His therapy consisted of a combination of diphenylhydantoin, phenobarbital and primidone.[a] Because of hyperkinetic behavior, administration of methylphenidate hydrochloride was begun. This produced a favorable quieting of behavior without sedation. In an attempt to produce greater improvement, the dose of methylphenidate hydrochloride was increased to 20 mg twice a day. Within a week ataxia was noticed. The methylphenidate dose was then reduced to 10 mg in the morning and 20 mg at night, whereupon ataxia subsided. Toward the end of therapy with methylphenidate, ataxia without nystagmus persisted. It was during this period that we first analyzed the serum samples collected previously and discontinued administration of methylphenidate. Plasma levels are given in Table I. This child was part of a study of interactions between phenobarbital and diphenylhydantoin in children.[1, 2] It is to be noted that this patient received relatively large doses of anticonvulsants.

TABLE I

ANTICONVULSANT LEVELS IN THE PRINCIPAL CASE

Period	Date	Serum Level,* mg/liter P	D	PB	Methylphenidate Hydrochloride Dose, AM/PM
Control	5/31/67	—	8.9	—	0/0
	6/21/67	4.4	8.6	23.0	0/0
Therapy with methylphenidate	7/26/67	2.8	11.2	28.3	10/10†
	8/31/67	8.1	26.0	39.0	20/20†
	9/ 6/67	21.5	28.6	34.4	10/20†
	10/ 4/67	20.8	23.0	34.0	10/20
	11/15/67	20.8	35.6	29.6	10/20
	12/13/67	9.9	29.9	37.2	10/20
	3/ 6/68	9.9	24.5	37.2	10/20
	4/ 3/68	9.9	33.4	35.0	0/0†
	4/15/68	16.5	34.0	35.0	0/0
	5/22/68	5.5	8.5	29.2	0/0

*P indicates primidone; D, diphenylhydantoin; and PB, phenobarbital. Diphenylhydantoin sodium (8.9 mg/kg/day) was given as a chewable tablet, and primidone (17.7 mg/kg/day) was in the form of a scored 250-mg tablet. Drugs were given in equal amounts daily. Serum samples were drawn between 1:30 and 3:30 PM.
†Dosage of drug started after serum was sampled on that day.
The daily dose of methylphenidate hydrochloride was changed as indicated.

[a] The interaction of the pair of drugs: phenobarbital and diphenylhydantoin had been initially investigated in adults and animals by two groups,[3, 4] [for review see Kutt,[5, 6] Cucinell,[7] Buchanan and Sholitan,[8] and Morselli *et al*,[9]]. Cucinell *et al* and Kutt *et al* had shown that the two drugs could interact in man as follows: (a) stimulation of metabolism of diphenylhydantoin by phenobarbital, (b) mutual inhibition of metabolism, and (c) no interaction. When this patient was studied information about such interactions in children was not available. The increase in the plasma levels of anticonvulsants appeared to be related to the coadministration of methylphenidate.[10]

Patient II. The patient is a 40-year-old black, male, a former U.S. soldier who had 15 years of active duty. His chief complaint was persistent hiccups which did not respond to tranquillizers or anticholinergic drugs. The Army physicians could not determine the cause of the hiccups. He was treated with methylphenidate hydrochloride, 10 mg orally q.i.d. without effect. Eventually it was found that this drug given i.v. (25 mg) was the only effective therapy.[b] During the remainder of his Army career he frequently entered the Emergency Service because of hiccups, which were successfully treated with intravenous methylphenidate. Despite the severity of the hiccups, he was returned to duty. Because of continued complaints that he could not work or eat, he was eventually discharged with a disability pension. He was treated at various Veterans Hospitals at numerous times. Despite several extensive workups no etiology was found for the hiccups. He continued to be treated with different sedatives, tranquillizers and antacids, etc., without relief, whereas i.v. methylphenidate was effective. The patient was admitted to the Clinical Pharmacology Service for evaluation in March, 1969. It was found that a few minutes after intravenous administration of methylphenidate, singultus ceased. Trials of placebo, amphetamines and oral methylphenidate were not successful. In subsequent months he was treated many times with intravenous methylphenidate at the Grady Hospital Emergency Service. In August, 1969, he was given methylphenidate intravenously 49 times. Since he was also being seen at the Veterans Administration Hospital and perhaps at other hospitals, it is not certain how many methylphenidate doses he received.

Because of such extensive usage, the question of "addiction" to methylphenidate was seriously considered and a psychiatric evaluation was undertaken. The psychiatrist suggested the possibility that the hiccups were feigned and a means to obtain the drug. During the course of these interviews the patient stated that he could differentiate intravenous methylphenidate from other agents because of the "rush" to his head produced by the drug. Since that time he was followed by the Psychiatric Service with moderate success, but he still frequently complained of hiccups. During this period it was also found that he was an alcoholic.

The patient's probable addiction to methylphenidate could have many causes, some of which could be interrelated: (1) the patient might have a genuine problem which was helped by methylphenidate, (2) he could have at one time been addicted to another drug and methylphenidate was a satisfactory alternate, and (3) the addiction could have been iatrogenically induced.

DRUG INTERACTIONS IN VIVO

Methylphenidate and Ethyl Biscoumacetate

We speculated that methylphenidate caused the rise of plasma levels in Patient I[10] by inhibiting drug-metabolizing enzymes. One of the reasons for this assumption was the structural similarity between the well-established inhibitor of drug metabolism, SKF-525A, amphetamine and methylphenidate (Fig. 1). At the time these studies were carried out, few drugs were known to inhibit drug metabolism in man.[5,6,11-15].

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[b] Although some authors indicate that methylphenidate is the agent of choice in singultus,[36] other workers disagree with this recommendation.[37] For the sake of simplicity methylphenidate hydrochloride (Ritalin[R]) will be referred to as methylphenidate.

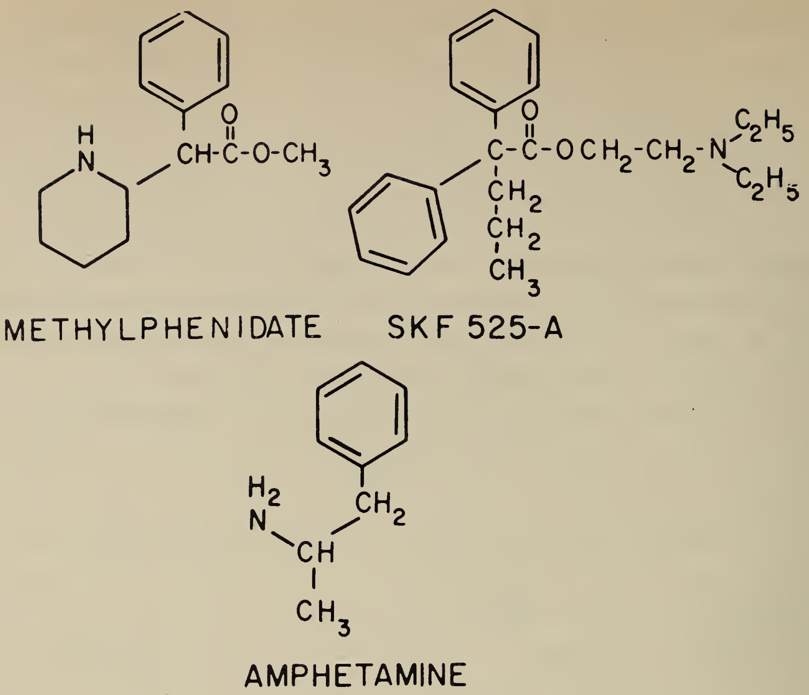


Figure 1 *Structures.*

After weighing the information obtained from Patient I we decided to test whether methylphenidate was indeed an inhibitor of drug metabolism in man. We chose ethyl biscoumacetate as the test drug in normal, male, informed volunteers because of the following considerations: 1. The pharmacologic effect of the anticoagulant could be abolished by administration of vitamin K_1. 2. Vitamin K_1 was known not to have an effect on the metabolism of coumarine anticoagulants.[16] 3. The analytical method was simple.[16] 4. The drug has dose-dependent characteristics;[16,17] we reasoned that a dose-dependent drug might be more susceptible to the effects of a weak inhibitor. 5. A large dose (20 mg/kg) could be given. 6. In previous studies[18] we had demonstrated effects of barbiturate pretreatment on ethyl biscoumacetate.[c] 7. Interactions involving binding to protein between methylphenidate and the coumarin were not expected because one compound is a base and the other an acid. 8. The drug is not appreciably excreted in urine in unchanged form.

A typical result is shown in Figure 2. Pretreatment with methylphenidate (10 mg b.i.d., p.o. 3 days) apparently prolonged the plasma level decline of ethyl biscoumacetate. One could argue that incomplete absorption might have affected the plasma levels. This possibility was ruled out because the peak levels were similar after ethyl biscoumacetate with or without pretreatment with methylphenidate. Besides this subject, several others were studied following the same protocol (Table II).[d] Based upon these observations we came to the tentative conclusion that methylphenidate was a potential inhibitor of drug metabolism in man. It would have been advantageous to give the coumarin intravenously rather than orally, but this was difficult under the Federal regulations.

[c] In fact, subsequent to our investigation, several groups have used coumarin anticoagulants as a test drug in man because of their importance and the fact that one can measure not only plasma levels but pharmacologic effects.[19-21]

[d] In one subject (No. 2) a long half-life was observed weeks after methylphenidate pretreatment. Since this was not observed in the other subjects, there may have been some extraneous cause affecting drug metabolism.

TABLE II

ETHYL BISCOUMACETATE HALF-LIVES IN METHYLPHENIDATE-TREATED ADULTS*

Subject (Age, Yr)	Days of Pretreatment With Methylphenidate**	Date	Peak Serum Level, mg/liter	Half-Life Hr
1 (34)	–	4/24†	170	3.5
	4	4/30	170	5.3
	–	4/31	172	6.5
	–	5/29	128	4.8
	–	8/13	130	4.0
2 (41)	–	5/10†	126	2.5
	3	5/13	131	6.0
	–	6/3	151	6.0
3 (26)	–	5/24†	160	2.3
	3	5/27	178	4.0
4 (34)	–	6/4†	112	2.0
	5	6/10	155	3.7
	–	7/5	118	2.0

*The dose of ethyl biscoumacetate was 20 mg/kg p.o.
**Methylphenidate hydrochloride was given orally, 10 mg twice daily.
†Control day.

The above-mentioned clinical studies were followed up by investigations of interactions between the pairs: methylphenidate and phenylbutazone and methylphenidate and imipramine. An important consideration in the choice of phenylbutazone and imipramine was the well-known fact that they are not excreted appreciably in urine in unchanged form.

Methylphenidate and Phenylbutazone[22]

We found that methylphenidate markedly inhibited the metabolism of phenylbutazone in mouse liver supernatant and microsomal preparations.[22] On this basis we proceeded with our studies in man.

Four normal, male, informed volunteers were given a single 400 mg oral dose of phenylbutazone and plasma levels of phenylbutazone were measured. The same experiment was repeated after pretreatment with methylphenidate (10 mg b.i.d. for 3 days). In one of these 4 volunteers there was a significant prolongation of the half-life of phenylbutazone.

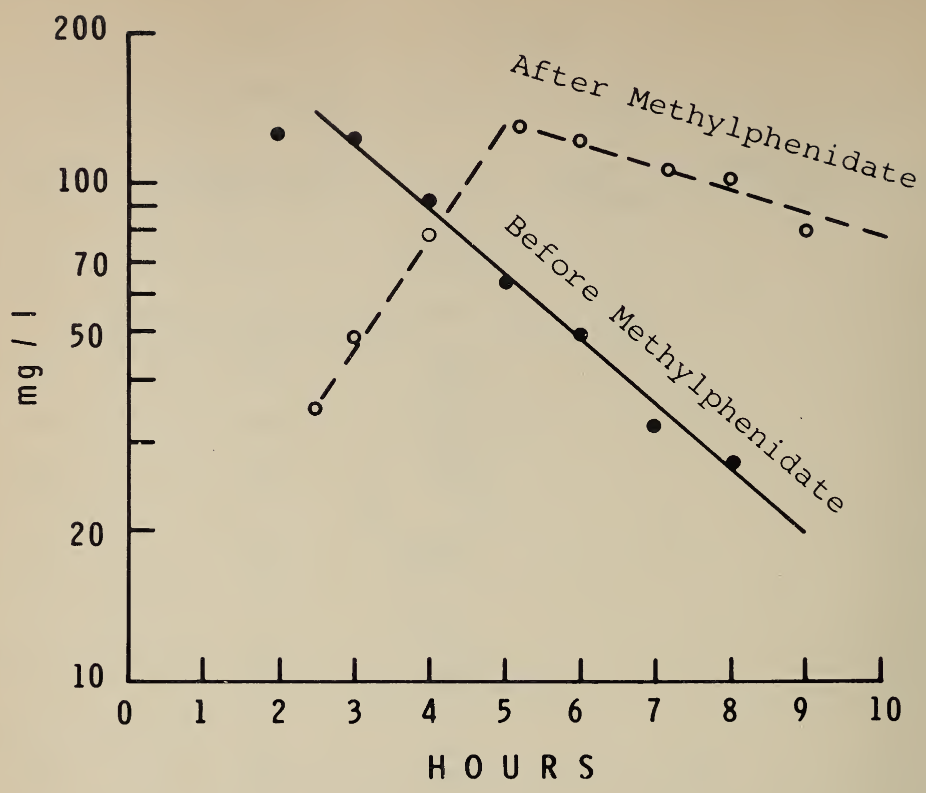


Figure 2 *Plasma levels of ethyl biscoumacetate. Dose 20 mg/kg p.o.*

When 6 patients on a chronic regimen of phenylbutazone (200-400 mg/day) were given methylphenidate, a significant increase in phenylbutazone plasma levels occurred in 5 of these subjects. The sixth subject was also receiving phenobarbital. The lack of effect in this patient could be rationalized on the basis of the known property of phenobarbital of stimulating drug metabolizing enzymes in man. In these patients not only plasma levels of phenylbutazone but also levels of oxyphenbutazone (a metabolite of phenylbutazone) were determined. Oxyphenbutazone plasma levels did not increase appreciably. This suggests that the inhibitory effect by methylphenidate is mainly on ring hydroxylation. On chronic dosage of phenylbutazone, the plasma levels of phenylbutazone were much higher than after a single dose. The observation that methylphenidate was more likely to inhibit metabolism when levels of another drug are relatively high, such as on chronic dosage, can be interpreted as follows. When drug metabolism is near saturation, a weak inhibitor of drug metabolism can produce an effect which it might not at a lower dosage. This hypothesis is supported by the fact that phenylbutazone metabolism *in vivo* is dose-dependent in animals and man.[17, 23] Individual variability of dose-dependence is considerable.[17, 23]

Methylphenidate and Imipramine

We initiated a study[24] to learn whether methylphenidate could inhibit the metabolism of imipramine in man under clinical conditions. The effect of methylphenidate was studied only after at least one week of imipramine administration. The reasons for this are as follows: 1. Imipramine exerts a therapeutic effect only after repeated and continuous administration. It appears that a certain amount of peripheral tissue loading is necessary before the uptake into the brain becomes therapeutically significant in man. 2. A steady plasma level of imipramine is reached only after at least one week.[25, 26]

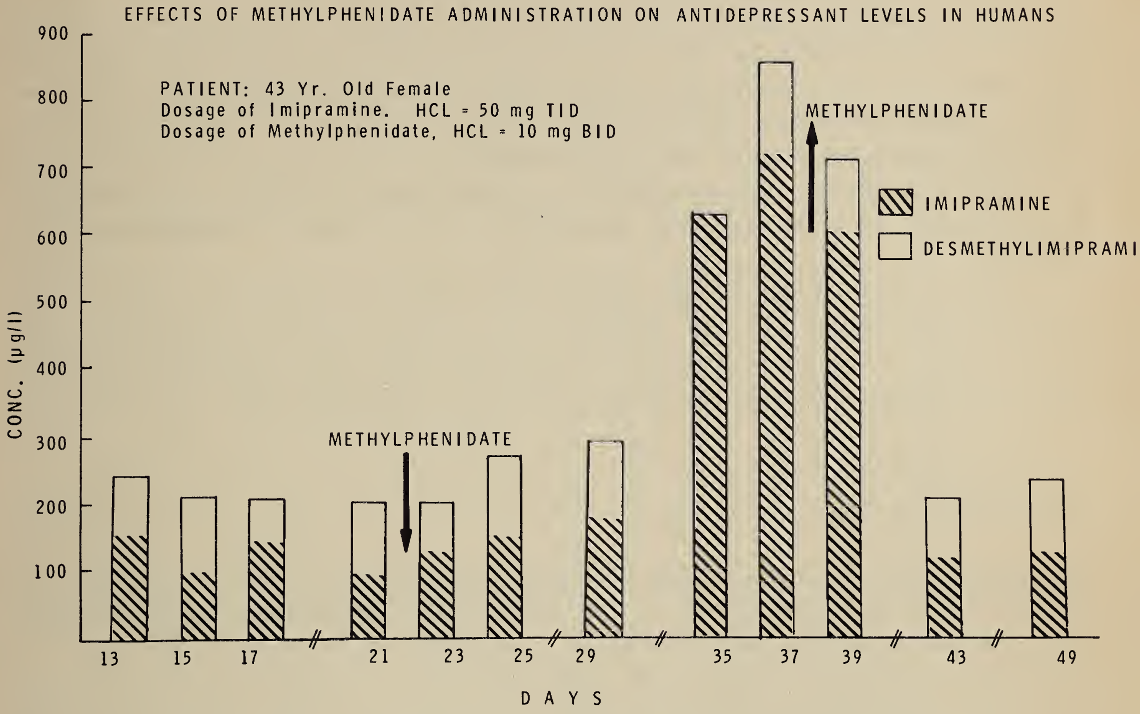


Figure 3 *Plasma levels of imipramine and desmethylimipramine. Arrows indicate start and end of methylphenidate treatment. Drugs were given orally.*

It was found that in several patients there was a dramatic increase not only of imipramine but also of desmethylimipramine plasma levels.[24] A typical result is shown in Figure 3. The effect is interpreted as one of inhibition for the following reasons: (a) the elevation of levels exhibited a slow onset (several days); (b) the effect wore off after several days; (c) there was no competition for plasma protein binding between methylphenidate and imipramine. Further confirmation was obtained by *in vitro* experiments with human liver fractions. Methylphenidate inhibited both hydroxylation and demethylation of imipramine.[24] Desmethylimipramine is further metabolized by the same enzyme system; these facts could explain the rise in plasma levels not only of imipramine but also of desmethylimipramine. Concomitant therapeutic improvement in several of the patients studied occurred (as measured by staff rating evaluations and psychiatric examination[26]). The patients selected for study had suffered from refractory recurrent depressions not amenable to shock treatment and/or imipramine alone.

Our observations were noted by other groups.[27-32] Though two groups confirmed our findings,[27, 32] other investigators disagreed with our observations.[28-30] Solow and Green[32] reported that in 3 cases diphenylhydantoin intoxication was caused by methylphenidate and was promptly reversed by discontinuing methylphenidate. An interaction between methylphenidate and imipramine was observed by Cooper and Simpson.[27]

One of the problems of evaluating the effect of methylphenidate on the metabolism of other drugs is that several types of drug interactions may take place simultaneously.[11] Thus it was shown that large doses of methylphenidate (5 mg/kg, i.v.) can alter the volume of distribution of thiopental (administered in a single dose) in dogs.[33] Presumably, thiopental assumed a smaller volume of distribution and this is probably due to the circulatory changes induced by methylphenidate. In man much lower doses (10 mg b.i.d. orally, pretreatment four days) has similar effects.[33] Thus the mechanism in these experiments is different than in the pair imipramine-methylphenidate, since the interaction is *transient.*

Drug interactions such as inhibition and stimulation of drug metabolism have been shown to be biphasic.[34] A clearer insight can, of course, be gained by *in vitro* studies.[10]

Still another difficulty in interpretation of drug interactions can be encountered, as indicated in our studies, of an interaction between allopurinol and probenecid.[35] It was shown that in one population allopurinol inhibited the metabolism of probenecid whereas in another population, the same effect was not found.

Metabolism

Observations on the two patients described (Patients I and II) led us to study the metabolism of methylphenidate. In the first case it was a question of understanding a drug interaction, in the second an apparent difference in response to oral versus i.v. doses. From previous information[11] it was clear that to interpret drug interactions one needs to have data on the metabolism and disposition of each drug involved, and even at times of their metabolites. In the literature on methylphenidate there was no information about the metabolism of the drug in man.

Metabolism of Methylphenidate in Man and Animals in vivo

The first study of distribution and elimination of carboxy-^{14}C labeled methylphenidate in rats was carried out in 1957.[38] These workers found that essentially all of the drug was rapidly excreted in urine and that there was practically no unchanged drug in urine. They gathered evidence for the presence of the deesterified metabolite (ritalinic acid), an unknown metabolite and its glucuronide. No $^{14}CO_2$ was excreted in the breath. After oral administration, ^{14}C was found in the brain and its concentration varied in different areas of the CNS.

Subsequently Sheppard and coworkers[39] studied the distribution of ^{14}C after intraperitoneal administration of carboxy-^{14}C methylphenidate to guinea pigs. They used solvent extraction methods to differentiate between methylphenidate and metabolites without identifying the latter. They found less than 2% of the administered radioactivity in feces. In tissue distribution studies the highest concentrations of ^{14}C were found in liver and kidney. Brain levels were low. In studies of subcellular distribution in liver, the highest concentrations were found in the supernatant fraction.

We gave carboxy-^{14}C methylphenidate intravenously to man (2.5 - 20 mg, 20 uc).[40] To separate methylphenidate from its major known metabolite, ritalinic acid, we used the following method. To 0.5 ml of plasma or urine, 1 ml 0.1 N NaOH and 20 ml methylene dichloride were added. After shaking and centrifugation, the organic phase was removed, evaporated to dryness and counted. Essentially no unchanged drug was found in urine, which agrees with the previous animal experiments. However, using gas chromatography, Schubert reported small amounts of methylphenidate (0.8 - 40 μg/ml) in urine of individuals driving while under the influence of large doses of methylphenidate.[41]

After i.v. administration, an initial distribution phase (T½ 0.5 - 1 hr) was observed followed by first order decline of methyphenidate-^{14}C in plasma (2 - 4 hr). This is much shorter than the 12 hour half-life observed for amphetamine.[42] The total ^{14}C in plasma after administration of methylphenidate-^{14}C rose several-fold in the first 2 hours and declined more slowly than methylphenidate. A similar pattern was observed by Dollery and associates for the drug propranolol.[43] These latter authors explained the phenomenon on the basis of formation of a metabolite. Our data also suggests that the concentration of metabolites circulating in plasma a few hours after administration is higher than that of the parent drug.

After oral administration of methylphenidate-^{14}C (20 mg), there was practically no circulating plasma level of unchanged drug. These observations could explain the difference in effect between intravenously and orally administered methylphenidate in Patient II. After an i.v. dose, methylphenidate could readily penetrate into the brain and other tissues.

One patient was given multiple doses of methylphenidate-^{14}C. Some accumulation of ^{14}C was observed (Fig. 4).

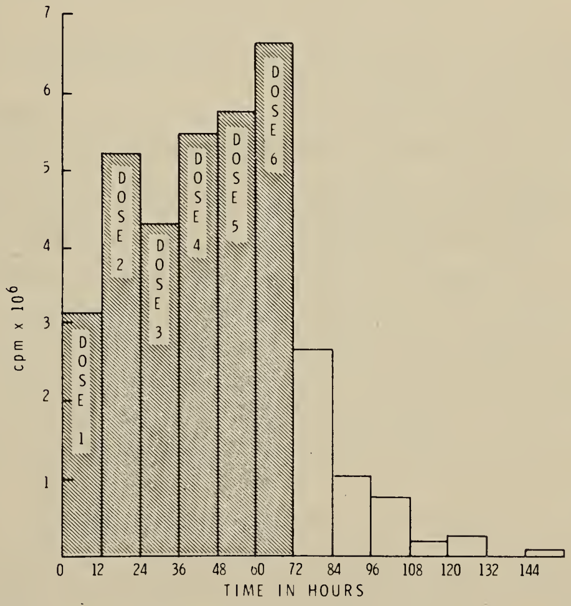


Figure 4 *Urinary excretion after multiple doses of methylphenidate-^{14}C. The patient had essential hypertension and gout (urate 10 mg%). The only other drug the patient received was 0.25 mg of digoxin qid, p.o. The subject was a 60-year old Negro male weighing 93 kg. Dose 1 (10 mg, 4 uc, carboxy methylphenidate-^{14}C) was given at 8 a.m. Studied in cooperation with Dr. S.A. Cucinell. 800 cpm = 1 ug methylphenidate equivalent.*

The above data indicated the need for identifying metabolites other than ritalinic acid, which is the major metabolite. While we were engaged in these experiments, Bartlett and Egger^{44} reported the presence in human and animal urine of oxo-ritalinic acid (Fig. 5). The mechanism of the formation of this metabolite is still speculative (see below). They found that in their subjects, the excretion of ritalinic acid was 50-81%; oxo-ritalinic acid accounted for 5-12%. In agreement with our findings, they reported less than 1% excreted as unchanged drug. We found 80-90% excreted as ritalinic acid and only 2-5% as oxo-ritalinic acid (the lower values of oxo-acid could have been due to decomposition since these urines were stored for a long time). We also found a small amount (2%) of p-hydroxy ritalinic acid (M-2; Fig. 5).

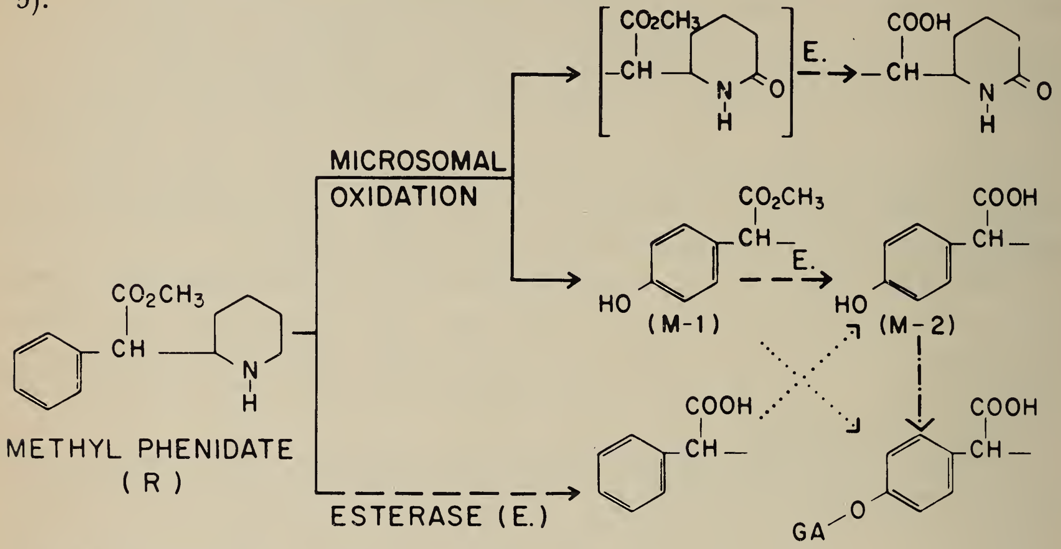


Figure 5 *Metabolism of methylphenidate. The metabolites on the top line are oxo-methylphenidate and oxo-ritalinic acid.*

Studies of the De-esterification of Methylphenidate-^{14}*C* in vitro

One ml of a solution of methylphenidate-^{14}C.HCl in distilled water was added to 0.5 ml of pH 7.4 Sörensen M/15 buffer, pH 7.4 0.2 M Tris-0.1 N HCl buffer, heparinized human plasma or serum to yield a final concentration of 1 x 10^{-8} – 2 x 10^{-4} M. The mixture was incubated for up to 24 hours in air at 37°. The extent of hydrolysis was determined by adding 1 ml of 0.1 N NaOH and extracting into 20 ml of mythylene dichloride. The lower phase was mixed with 0.5 ml of 0.05 N HCl and the organic solvent was evaporated (air). Counting fluid (18 ml) was added to the residue and ^{14}C was determined (Beckman LS-255 scintillation spectrometer, counting efficiency 90%). The half-life of de-esterification (T½) of methylphenidate by plasma, serum or buffer was the same (11.5 hr; range 9.0 – 14.3 hr). There was no effect of the concentration of methylphenidate on hydrolysis.

The hydrolysis of methylphenidate-^{14}C.HCl was shown to be highly pH dependent. Methylphenidate-^{14}C.HCl (final concentration 2 x 10^{-4} M) was incubated at 37° for up to 24 hours in Sörensen's disodium citrate buffer at pH's 1.3, 2 and 4 and Sörensen's phosphate buffer at pH's 6.0, 7.0, 7.2, 7.4, 7.6, 7.8 and 8.0. Methylphenidate was found to be stable at pH 1.3-4.0 for up to 24 hours and exhibited T½'s of 113, 25, 15, 11, 6.3, 5.0 and 3.7 hours, respectively, at pH's 6.0, 7.0, 7.2, 7.4, 7.6, 7.8 and 8.0.

New Metabolites of Methylphenidate[45]

Since p-hydroxy methylphenidate (m-1; Figure 5) and its acid (M-2) were likely metabolites of methylphenidate, these compounds were synthesized. We prepared them by condensation of p-methoxybenzyl cyanide with α-bromo-pyridine in the presence of sodium amide. Methanolysis of the resulting nitrile, catalytic reduction of the pyridine ring and treatment with HBr gave M-2 as a mixutre of *erythro* and *threo* forms; esterification of M-2 gave M-1.

Urine from man, dog and rat given methylphenidate-^{14}C was extracted with ethyl acetate under basic conditions (saturated K_2CO_3). The aqueous phase was made acidic (HC1) and extracted with n-butanol. The presence of conjugates was evaluated in the remaining aqueous phase by incubation with β-glucuronidase and sulfatase. Thin-layer chromatography of the extracts indicated zones corresponding to ritalinic acid, M-1, M-2, oxo-ritalinic acid, oxo-methylphenidate and still unidentified metabolites. In man and dog aromatic hydroxylation represented a minor fraction of the dose, whereas in the rat it was the major pathway.

Sufficient quantities of M-1 were isolated from rat urine and subjected to mass spectral analysis (in cooperation with the Research Triangle Institute Mass Spectrometry Center). The fragmentation patterns of the synthetic and isolated material were identical.

Furthermore, *in vitro* experiments with rat liver 9000 x g supernatant and microsomal fractions indicated formation of ritalinic acid, M-1, M-2, the glucuronide of M-2, oxo-ritalinic acid and oxo-methylphenidate. Experiments are underway to evaluate the pharmacological activity and entry into the brain of M-1.

Speculation Concerning Methylphenidate Metabolites

By analogy with amphetamines certain predictions can be made regarding the probable pharmacological effect, in particular the inhibition of the uptake of norepinephrine. The ring-hydroxy metabolite of amphetamine had different activity than the side-chain metabolite.[46, 47] Beta-hydroxy amphetamine had about 1/5 the activity of amphetamine, whereas para-hydroxy amphetamine was about 3 times as active as amphetamine (as an inhibitor of neuronal uptake of norepinephrine). This suggests that the para-hydroxy metabolite of methylphenidate could be more potent than methylphenidate, whereas the oxo-metabolite would be less active. However, *in vivo* effects may not follow this pattern because of biological transport, excretion, etc. Since metaraminol is more active than amphetamine, other metabolites of methylphenidate such as a possible meta-hydroxy or catechol metabolite might be very active.

A contrast exists between methylphenidate and amphetamine in terms of metabolism. Whereas aromatic hydroxylation of amphetamine could take place *in vivo*[48] and rat liver slices,[49] rat liver microsomes could not metabolize amphetamine.[49] On the other hand, rat liver microsomes were able to catalyze the aromatic hydroxylation of methylphenidate. Further, as ritalinic acid is an analog of phenylacetic acid and the latter is known to form glutamine conjugates,[50] several glutamine conjugates of methylphenidate metabolites are conceivable.

Oxo metabolites of methylphenidate are formed by the oxidation of the carbon alpha to the nitrogen of the piperidyl ring. Several mechanisms are possible (Fig.

6). The formation of similar metabolites via ring opening followed by closure, may be a fairly common occurrence. Such mechanisms have been suggested in the formation of cyclized metabolites from a number of drugs: tremorine,[51] nicotine,[52, 53] mefruside,[54] prolintane,[55] mepivacaine,[56] cyclophosphamide,[53, 57] diphenidol,[58] medazepam,[59] etc. In the case of cyclophosphamide, tremorine and nicotine, the open ring precursor to the cyclized metabolite has been identified and isolated.

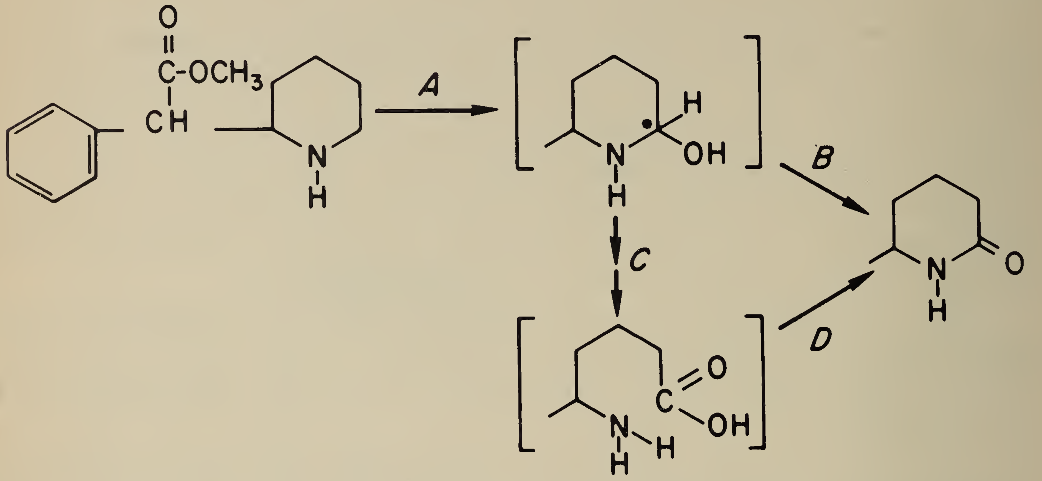


Figure 6 *Proposed mechanism for the formation of the oxo-metabolite of methylphenidate. A = microsomal oxidation, B = dehydrogenation, C = enzymatic oxidation. This may involve the formation of a Schiff base by dehydration, formation of an aldehyde followed by oxidation to an acid by aldehyde dehydrogenase. D = ring closure by dehydration.*

The formation of cyclized metabolites can also take place by other routes.[e] One of these involves epoxides of aromatic compounds formed by the oxidation of aromatic and olefinic compounds—it can also be considered as a three-membered ring metabolite. Such a mechanism is probably responsible for the formation of the p-hydroxy metabolite of methylphenidate.[66, 67]

DRUG INTERACTIONS IN VITRO

We resorted to *in vitro* experiments to understand the interactions observed with patient I.[10] In these preliminary experiments with human and mouse liver preparations, we found that methylphenidate inhibited zoxazolamine hydroxylase. With human liver, the inhibition was equipotent with that found with SKF-525A, whereas in mouse liver methylphenidate was less potent. Using different species we later reported[68] that this inhibition was fairly general. (The test reactions were oxidation of phenylbutazone, demethylation of aminopyrine and glucuronidation of ortho-amino-phenol. The cofactor additions used were those employed by Kuntzman *et al.*[69]) Detailed enzymatic kinetic studies of the inhibition of the metabolism of diphenylhydantoin by methylphenidate and other related inhibitors are reported herewith.

[e] Some of these are following oxidation (a) enzymatic and non-enzymatic intramolecular cyclization (methadone[60] and lidocaine,[61]) and (b) condensation with another compound or its metabolites,[62, 63] acetylation followed by cyclization (hydralazine[64], and hydrolysis of a ring compound followed by the formation of a different cyclized product (penicillins[65]).)

In vitro *Studies with Diphenylhydantoin*

A 10% (w/v) rat liver homogenate was prepared with cold (0-5°) pH 7.4 potassium phosphate buffer (0.2 M). Four ml of the 9000 x g supernatant was used in each incubation mixture (equivalent to 400 mg of tissue). The cofactor mix used was the same as described by Kutt and Verebely[70], the overall final volume also being 5 ml. The mixtures were incubated in air at 37° with shaking for 15 min, at which point 0.5 ml of 2.5 N NaOH was added to terminate the reactions. At least 5 concentrations of 5,5-diphenylhydantoin-4-^{14}C (specific activity 4.7 mCi/mmole, New England Nuclear Corp. or Schwarz/Mann) ranging from 4×10^{-6}M to 1.5×10^{-4}M were used. The inhibitors were added in concentrations ranging from 6×10^{-5}M to 1×10^{-3}M. The microsomal protein concentrations of the incubation system were determined by the method of Sutherland *et al.*[71] as modified by Robson *et al.*[72], with bovine serum albumin as a standard.

Purity of the tracer was established by thin-layer chromatography (see below). The analysis was carried out by a method similar to that used for dog plasma.[11] When high concentrations of label were used (0.5 uc/flask) 1 ml of incubation mixture was diluted with 3 ml of M pH 7.0 sodium phosphate buffer. One ml of the dilution was placed in a flask with 4.5 ml of the buffer and 15 ml of chloroform and shaken for 10 minutes. The chloroform was removed and the aqueous phase was reextracted with 15 ml of fresh chloroform. Three ml aliquots of each organic phase were pooled (total = 6 ml) and evaporated to dryness under N_2 in a scintillation vial, after which the scintillation mixture was added and counted. One ml of the aqueous phase was added to the scintillation solution and allowed to stand overnight prior to counting.

Thin-layer chromatographic methods were utilized for proof of specificity as well as the determination of the principal metabolic product, 5-phenyl, 5'-p-hydroxyphenylhydantoin in the organic extracts. After pooling the chloroform extracts, a 6 ml aliquot was evaporated to dryness under N_2; the residue was redissolved with 95% ethanol and suitable aliquots (50-100 λ) were spotted on silica gel G (0.25 mm) glass plates. Lipid-like materials were removed prior to exposure to the developing solvent by placing the plates in chloroform and allowing this solvent to travel for at least 10 cm. After air-drying, the plates were developed in solvent system A – isobutanol:chloroform:ammonium hydroxide (72:48:6 v/v) and 1 cm areas were scraped and counted. In this system, the R_f for diphenylhydantoin and its p-hydroxy metabolite (Aldrich Chemical Corp.) were 0.75 and 0.40, respectively. Suitable individual standards for these compounds were concurrently run in parallel. Visualization for the former was effected with mercuric sulfate reagent[73], while for the latter 1% iodine in methanol was used.

The p-hydroxy metabolite remaining in the aqueous phase after chloroform extractions was determined as follows. The aqueous phases (3 ml) were each extracted with 4 x 10 ml of ethyl acetate. After evaporation with N_2 at room temperature, the residues were redissolved in ethyl acetate; suitable aliquots (50-100 λ) were chromatographed in solvent system A.

Studies of specificity were carried out with incubations containing diphenylhydantoin alone and in the presence of inhibitors. It was found that the chloroform extract consisted mostly of the parent compound. About 30% of the p-hydroxy metabolite formed was co-extracted. The aqueous phase contained this metabolite and small amounts of other polar metabolites (<5%). Correction factors were determined for the amounts of p-hydroxy metabolite in both phases based on thin-layer chromatographic results. The enzyme kinetics were based on the rate of appearance of p-hydroxy diphenylhydantoin.

The effects of *threo*-methylphenidate, *erythro*-methylphenidate and ethylphenidate on the metabolism of diphenylhydantoin were studied in the microsomal preparations. These compounds were obtained through the courtesy of Ciba-Geigy. In addition, SKF-525A (2×10^{-7}M to 1×10^{-4}M) was also compared in the same systems. The enzyme kinetics were first examined by means of Lineweaver-Burk plots and the constants K_m and V_{max} were determined by the use of Hofstee-type graphs. The values of K_i were graphically obtained from Dixon-type graphs.

The formation of the p-hydroxylphenyl metabolite was shown to be linear for at least 15 minutes. By our thin-layer chromatographic procedures quantitative analytical data were obtained. Thus, we accounted for the p-hydroxyphenyl metabolite remaining both in the aqueous and organic extracts.

The hydroxylation of diphenylhydantoin was competitively inhibited by methylphenidate (Table IV), the K_i values being about 2×10^{-4}M. Ethylphenidate and *erythro*-methylphenidate, analogs with lesser central nervous system activity, showed equipotent inhibitory action. In agreement with previous findings, SKF-525A appears to be a strong non-competitive inhibitor of aromatic hydroxylation, the K_i being approximately 6×10^{-5}M.

It is of interest that methylphenidate inhibits aromatic hydroxylation to a greater extent than other metabolic pathways. Thus, with substrates such as chlorpromazine and imipramine the K_i values have been reported to be 8×10^{-5}M and 7×10^{-4} M, respectively.[74] In contrast with methylphenidate, amphetamine is a much weaker inhibitor of hexobarbital metabolism; 50% inhibition requires 10^{-2} to 10^{-3}M concentrations.[75]

The above *in vitro* studies do not prove inhibition of drug metabolism in man. In man, concentrations of methylphenidate we now know are much lower than those employed in the *in vitro* studies. However, in a patient conditions conceivably could be produced in which methylphenidate would affect drug metabolism (e.g., presence of other drugs, cumulation of metabolites, lower P450 levels).

Selected Physicochemical Properties which Influence Metabolism and Distribution of Methylphenidate

The pK_a of methylphenidate (presumably of the *threo* racemate) was found to be 8.8[f].[76] Schubert[41] reported the 'pK' of the *threo* and *erythro* ritalinic acids (hydrochlorides) as 7.6 and 6.6, respectively.[g]

In our laboratory we determined the relative lipid solubility of methylphenidate and its metabolites (measured by partitioning between pH 7.4 Sörensen buffer and various organic solvents). The order was as follows: methylphenidate, oxo-methylphenidate $>$ M-1 $\gg$ ritalinic acid $>$ oxo-ritalinic acid $>$ M-2.

Methylphenidate and its metabolites were poorly bound to human albumin (equilibrium dialysis, unpublished experiments).

[f] Neither the solvent nor the experimental conditions were given.

[g] Schubert[41] did not give the experimental condition and he also incorrectly quoted the pKa reported by Siegel *et al*[76] as 0.8, for methylphenidate.

TABLE III

HYDROLYSIS OF METHYLPHENIDATE BY HUMAN AND ANIMAL TISSUE PREPARATIONS[a]

Species	Tissue	Mean T½ of Hydrolysis (hours)
Man	Liver[b]	3.0
Baboon	Liver[b,c]	1.0
Mouse	Liver[d]	1.7
Mouse	Plasma[e]	1.8
Rabbit	Liver[d]	< 0.5
Rabbit	Intestine[f]	14

[a] All incubations were carried out in air at 37°.

[b] 2.0 ml of 10% liver homogenate (in 1.15% KCl) was adjusted to a final volume of 6.0 ml with pH 7.4 Sörensen phosphate buffer. Methylphenidate-^{14}C was added so that the final concentration was 1.0×10^{-4} M.

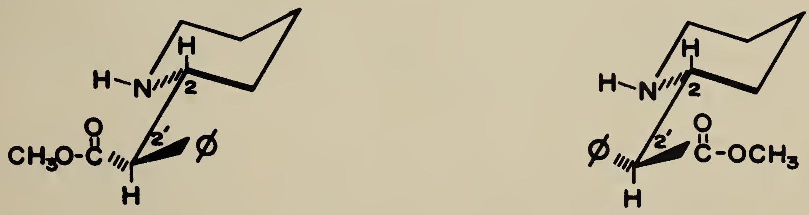
[c] Rhesus liver behaved in the same manner. Monkey liver samples were obtained through the cooperation of Dr. H.M. McClure of the Yerkes Primate Center, Emory University.

[d] 1.0 ml of 20% liver homogenate (in pH 7.4 Sörensen phosphate buffer) was incubated with 0.2 ml of a solution of methylphenidate-^{14}C (final concentration 2×10^{-4} M, pH 7.3 - 7.4).

[e] Plasma was diluted 1:1 with pH 7.4 Sörensen phosphate buffer; incubation was carried out as in [d].

[f] 10% intestinal homogenate (pH 7.4) was used in the procedure described in [d]. The value of 14 hours for T½ is about the same as that in buffer at pH 7.4.

Siegel *et al*[76] studied the rate of hydrolysis of methylphenidate under unphysiologic conditions (50-80°, pH 1.1-6.1), and found two different mechanisms operating[h].



[2R:2'R,(+)] threo Methylphenidate

[2R:2'S,(+)] erythro Methylphenidate

Figure 7 *Structures. 2 and 2' indicate the position of asymmetric centers. Thick lines represent bonds above the plane; crosshatched lines, below the plane. A hydrogen bond has been proposed between the carbonyl oxygen and a proton attached to the nitrogen of the* threo *form of methylphenidate.*

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[h] At 80°C (pH 3.0 and 6.0) we found similar hydrolysis rates.

TABLE IV

THE INHIBITION OF THE HYDROXYLATION OF DIPHENYLHYDANTOIN[a]

Inhibitor	V_{max}[b] umol/g-min (x 10^{-3})	K_i (M x 10^{-4})
–	9.1 ± 0.6	–
Threo- or *Erythro-* Methylphenidate[c]	8.9 ± 0.8	2.1 ± 0.4
Ethylphenidate[c]	8.5 ± 0.4	1.8 ± 0.3

[a] Measurement of conversion to 5-phenyl-5'-parahydroxy-phenylhydantoin with rat liver 9000 x g supernatant. Concentrations of diphenylhydantoin used (M) 4.0 x 10^{-6}; 2.0 x 10^{-5}; 3.8 x 10^{-5}; 7.5 x 10^{-5}; 1.5 x 10^{-4}. The amount of protein in each incubation mixture was 30 ± 4 mg.

[b] Km for control system: 2.7 x 10^{-5} M ± 0.3 All data expressed as mean ± S.E. are results of 5 experiments.

[c] Inhibitor concentrations (M): 6 x 10^{-5}; 6 x 10^{-4}; 1 x 10^{-3}.

The above-mentioned properties are likely to influence the distribution and metabolism of methylphenidate. By extrapolating Siegel *et al's* data[76] to 37° and from our de-esterification studies (cited above) one can assume that methylphenidate is stable in the stomach. Further, since it will be completely protonated at acid pH, it will not be appreciably absorbed from the stomach. The stability of methylphenidate at pH 3 was attributed to hydrogen bonding.[76] The authors did not specify that their hypothesis was restricted to the *threo* form only; the *erythro* analog will not readily form intramolecular hydrogen bonds (Fig 7).

The reported 'pK' of ritalinic acid isomers do not appear to be correct. Of the two isomers, the *threo* racemate should have a lower pKa because of hydrogen bonding. Nevertheless if the pKa of the racemate of *threo-* ritalinic acid (the major metabolite of *threo*-methylphenidate) is near physiologic pH, its renal clearance would be affected by urine pH. Based on their lipid solubility, only methylphenidate, oxo-methylphenidate and M-1 should readily enter the brain. The polarity and low binding of ritalinic acid and its oxo- and hydroxy- analogs probably are responsible for their rapid renal clearance. The polarity of ritalinic acid in turn is probably responsible for its limited further metabolism.

Note added in proof: The data reported in abstract form[40,45] have been published as a full paper.[79] The phenomenon of an increase of plasma levels of radioactivity after i.v. adminsitration of labeled drug is not unique to methylphenidate and propranolol,[43] but also occurs for haloperidol.[80]

ACKNOWLEDGEMENTS

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Effect of Diazepam on Sulfobromophthalein Excretion

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ABSTRACT Metabolites of diazepam are excreted into the bile of rats as glucuronide conjugates. Experiments were carried out to see if sulfobromophthalein (BSP) interfered with the biliary excretion of diazepam metabolites (measured as benzophenones). In control rats, 25 to 50% of the administered dose of diazepam (50-300 mg/kg, i.p.) was excreted as metabolites within 4 hours. A dose-related excretion pattern was observed. BSP administration (60 or 120 mg/kg, i.v.) 1 hour after diazepam resulted in a marked dose-related diminution in the biliary excretion of diazepam metabolites.

The effect of varying doses of diazepam (50-300 mg/kg, i.p.) on the biliary excretion of BSP (60 or 120 mg/kg, i.v.) was also studied. The diazepam was given 1 hour before the BSP. Diazepam treatment resulted in a marked dose-dependent diminution in biliary excretion of BSP. The effect on BSP excretion was more striking when the higher dose (120 mg/kg) of BSP was utilized. The peak biliary concentrations of BSP were markedly reduced, suggesting that diazepam decreased the hepatic excretory capacity for BSP elimination. Diazepam (150 mg/kg) decreased the biliary Tm for BSP, but not for BSP-glutathione. Plasma BSP retention was observed in rats treated with varying doses of diazepam (50-300 mg/kg, i.p.). Retention of BSP-glutathione, however, did not occur with diazepam treatment (100 mg/kg, i.p.). The data suggest that diazepam may affect several steps involved in BSP elimination and perhaps not only the final excretory step.

Within the last ten years, there has been considerable interest in the role of the bile in the excretion of chemicals.[1, 2] A number of substances are thought to be transported from the parenchymal cell into the bile, although the mechanism of transport has not been elucidated in any of these cases. It appears that the transport of these substances across the biliary epithelium into bile requires an active secretory process because (1) a bile to plasma ratio exceeding 10 is observed with many substances; (2) as the dose given to the animal increases, excretion into the bile seems to reach a plateau value; (3) substances with similar chemical characteristics can enter into competition for biliary excretion; and (4) metabolic inhibitors can inhibit the secretion of some of these substances. It appears that a separate transport system exists

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for anionic substances, for cationic compounds, and for nonionic substances.[1, 3]

Molecular weight can influence the biliary excretion of chemicals.[4, 5, 6] In the rat, an anionic substance requires a molecular weight greater than 300 in order to be excreted into the bile. Marked species differences occur with substances having molecular weights between 300 and 500. The rat, dog, bat and hen excrete these substances into the bile more extensively than do the rabbit, guinea pig and rhesus monkey; the cat and sheep seem to be intermediate.[6, 7] With compounds having molecular weights between 500 and 1000, species variation is less and biliary excretion of these substances exceeds 40 per cent of the administered dose.[2] Since many substances are conjugated with glucuronic acid, it has been shown that this conjugation step will increase the molecular weight to the point where many substances can be excreted into the bile.

The excretory characteristics for the dye sulfobromophthalein (BSP) have been studied extensively. This substance is conjugated with glutathione before its excretion into the bile and is excreted into the bile by an anionic transport system.[1, 2]

Diazepam is a substance which is currently used extensively in medical practice. It has been shown that this material is rapidly metabolized in the liver and conjugated with glucuronic acid.[8, 9, 10] The resulting metabolites would all have molecular weights larger than 300 and would possess an anionic configuration. Therefore, it is theoretically possible that diazepam could be excreted into the bile in considerable quantities and should be transported by the anionic transport system proposed for biliary excretion.

Besner *et al.*[11] have demonstrated that in the rat diazepam itself is not excreted into the bile; after hydrolysis of bile with β-glucuronidase, 3-hydroxydiazepam, 4'-hydroxydesmethyldiazepam, traces of oxazepam, as well as two unidentified metabolites, were detected. One purpose of the present study was to see if the metabolites of diazepam were indeed excreted in large quantities in the bile of rats.

Since anionic substances have been found to compete with one another for excretion into bile, it was of interest to see whether diazepam and its metabolites would compete with BSP for biliary excretion. In isolated perfused rat livers, Kvetina *et al.*[12] have shown that diazepam decreases the disappearance rate of BSP. A second purpose of the present study was to investigate the effect of diazepam on the biliary excretion of BSP and also the effect of BSP on the biliary excretion of the metabolites of diazepam.

METHODS

In the experiments to be described, Sprague-Dawley rats weighing between 250 and 325 grams were employed. The animals were anaesthetized with pentobarbital sodium (45 mg/kg), a laparatomy was performed and the common bile duct was cannulated with PE-10 tubing. The abdomen of the animal was then closed with wound clips and anaesthesia continued for the duration of the experiment. Body temperature was maintained at 37°C for the duration of the experiment to prevent hypothermic effects on biliary excretion.[13]

BSP was given intravenously (60 or 120 mg/kg). Bile was collected at 15-minute intervals for 1.25 hours. The BSP content in 50-μl aliquots was determined by dilution with 0.01 N NaOH; the absorbance was determined using a Coleman Junior

II spectrophotometer at 575 nm.

The diazepam was given intraperitoneally (50, 100, 150 or 300 mg/kg) 1 hour before the BSP. The diazepam was suspended in carboxymethylcellulose and propylene glycol to yield a suspension of 50 mg diazepam/ml. Control rats received the vehicles only. Bile was collected at 1-hour intervals for 4 hours.

For analysis of the diazepam metabolites, these were converted to benzophenones and the technique of Besner *et al.*[11] was employed: the bile (about 1 ml) was hydrolyzed with 6N HCl (10 ml acid for 1 ml of bile) for 1 hour at 100°C; the pH was then adjusted to 7.3 with 12N NaOH; the cooled solution was extracted twice with about 30 ml of anhydrous diethyl ether; the organic phase was removed and dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The residue was dissolved in absolute ethanol and the total benzophenone content of each sample was determined by ultraviolet spectrophotometry using an Unicam SP-500 at 232 nm.

RESULTS

Biliary Excretion of Diazepam Metabolites

In this series of experiments, four separate doses of diazepam (50, 100, 150 or 300 mg/kg) were used. The inhibitory effects of two separate doses of BSP (60 or 120 mg/kg) were assessed since it was of interest to see whether BSP affected the biliary excretion of diazepam metabolites. The benzophenones were measured in four consecutive 1-hour periods. In all experiments, the BSP was given 1 hour after the diazepam.

Figure 1 depicts the effect of BSP on the excretion of the metabolites when 50 mg/kg of diazepam was given to the rats. The data are expressed on a mg/kg body weight basis. Since each collection period was analyzed separately, the values given in the figure represent the amount of metabolites excreted per hour.

With 50 mg/kg of diazepam, the maximum rate of metabolite excretion in the bile occurred 1 to 2 hours after the administration of diazepam to control rats. At 4 hours, excretion was still going on. When BSP was given intravenously, there was a marked diminution in the amount of metabolites excreted in the bile at 2 hours; however, by 4 hours the excretion was normal. Both doses of BSP were able to reduce the amounts of metabolites excreted but the 120 mg/kg dose had a more profound and a longer lasting effect.

When the dose of diazepam was increased to 100 mg/kg, it was found that the low dose of BSP did not have as marked an effect on the excretion of metabolites as seen previously (Figure 2). However, at 2 hours, the value was still significantly lower than in control rats and had returned to normal by 3 hours. When 120 mg/kg of BSP was administered, however, the depression in benzophenones measured was still evident at 2, 3 and 4 hours.

When 150 mg/kg of diazepam was administered, the biliary excretion rate of metabolites increased to a peak value of about 15 mg/kg/hour (Figure 3). Both doses of BSP reduced the excretion rate of diazepam to a value which was about 33% of the control rate at 2 hours; by 4 hours, the effect appeared to be gone.

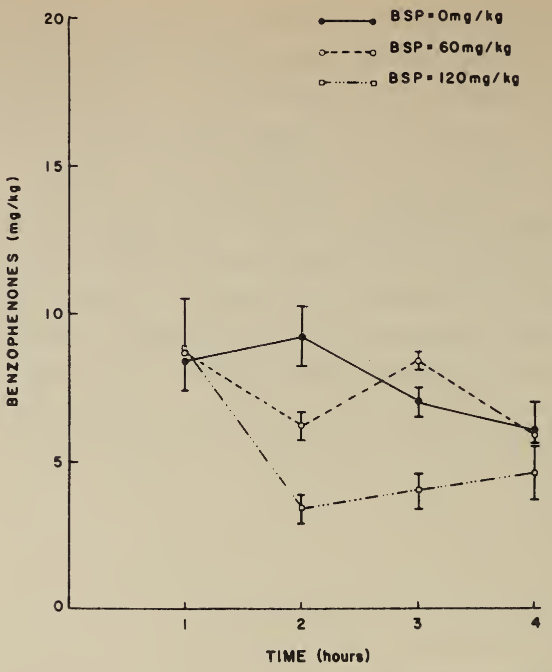

Figure 1: *Effect of BSP on the biliary excretion of diazepam metabolites after administration of diazepam (50 mg/kg, i.p.). The diazepam was given 1 hour before the BSP. Each point is the mean ± SE of 4 rats. The diazepam was given at 0 hour.*

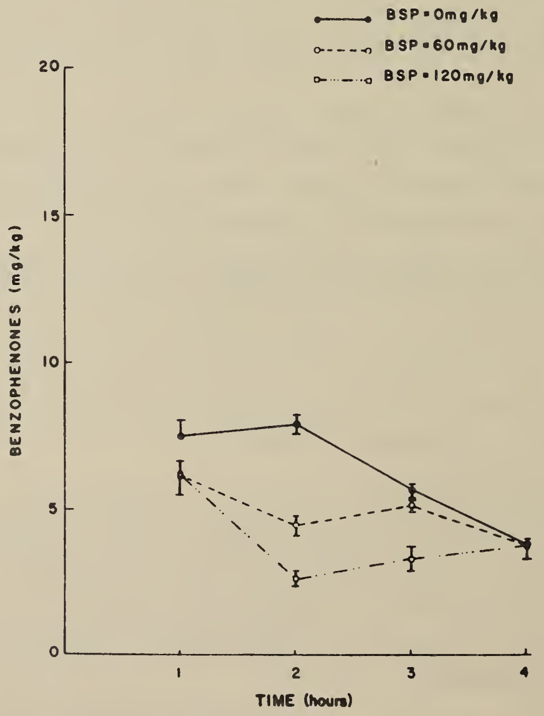

Figure 2: *Effect of BSP on the biliary excretion of diazepam metabolites after administration of diazepam (100 mg/kg, i.p.). The diazepam was given 1 hour before the BSP. Each point is the mean ± SE of 4 rats. The diazepam was given at 0 hour.*

With 300 mg/kg of diazepam, the peak excretion rate was over 20 mg/kg/hour at 2 hours in control rats (Figure 4). However, BSP was still capable of significantly decreasing the biliary excretion of diazepam metabolites. At 2 hours, the 60 mg/kg dose of BSP reduced the rate by about 66% and the 120 mg/kg dose reduced it by more than 75%.

The effect of BSP on benzophenone concentration is illustrated in Figures 5 and 6. Both doses of BSP significantly reduced the concentration of benzophenone measured 2 and 3 hours after administration of diazepam.

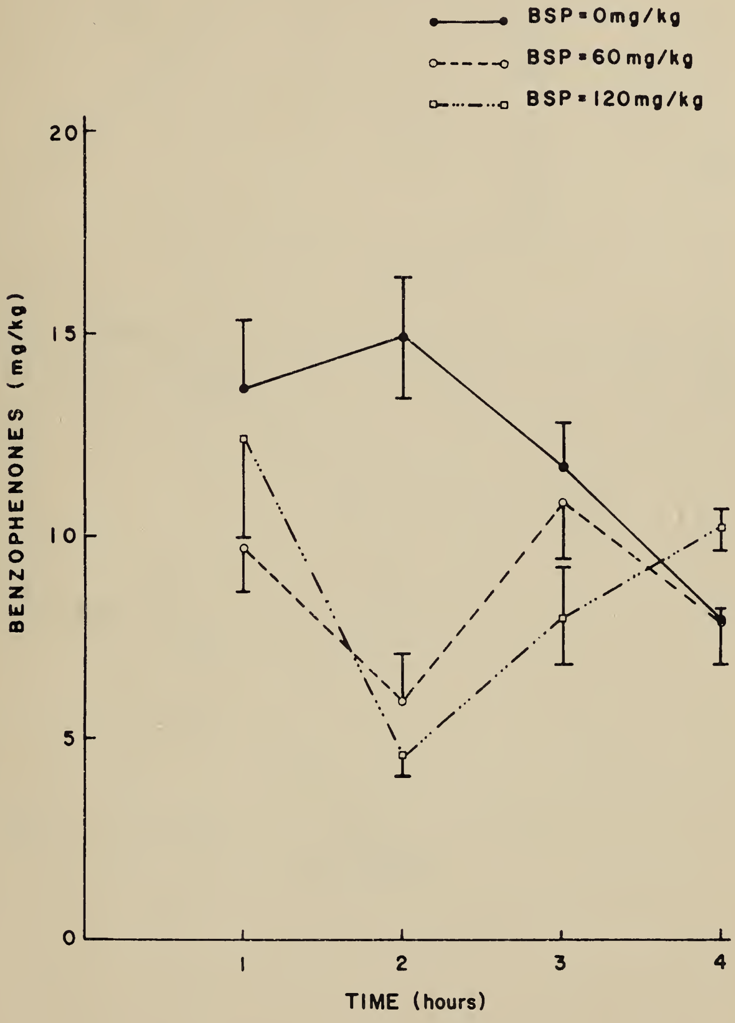

Figure 3: *Effect of BSP on the biliary excretion of diazepam metabolites after administration of diazepam (150 mg/kg, i.p.). The diazepam was given 1 hour before the BSP. Each point is the mean ± SE of 4 rats. The diazepam was given at 0 hour.*

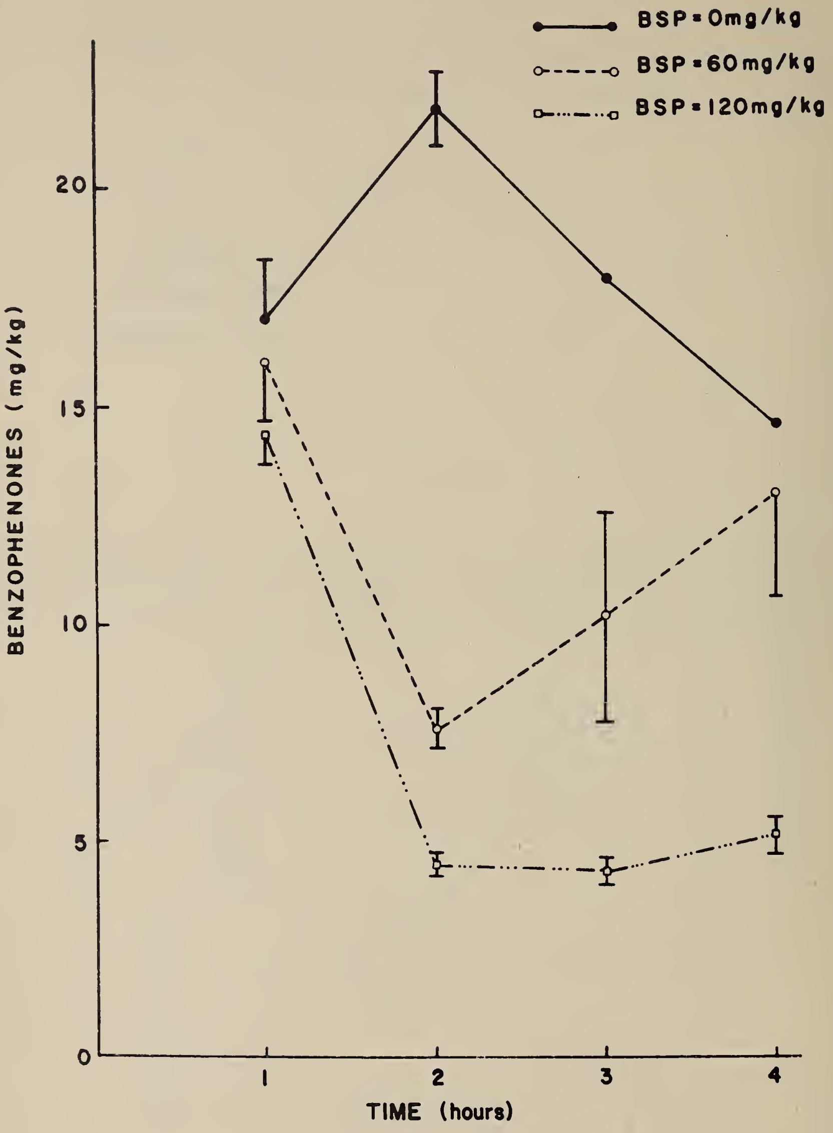

Figure 4: *Effect of BSP on the biliary excretion of diazepam metabolites after administration of diazepam (300 mg/kg, i.p.). The diazepam was given 1 hour before the BSP. Each point is the mean ± SE of 4 rats The diazepam was given at 0 hour.*

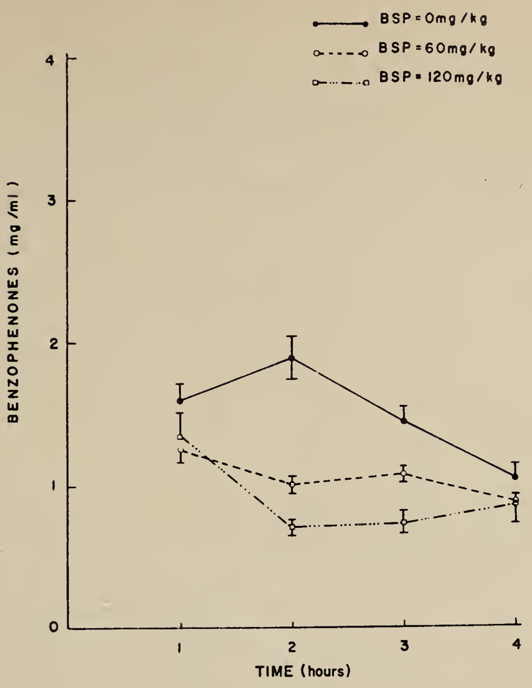

Figure 5: *Effect of BSP on the biliary concentration of benzophenones after administration of diazepam (50 mg/kg, i.p.). The diazepam was given 1 hour before the BSP. Each point is the mean ± SE of 4 rats. The diazepam was given at 0 hour.*

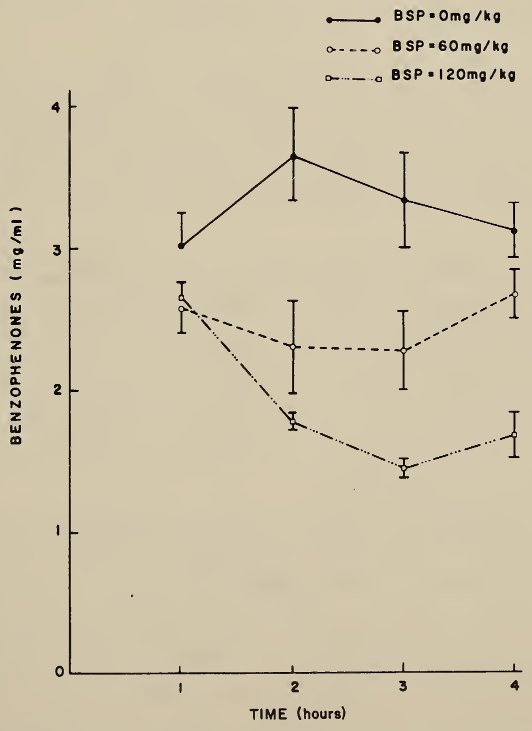

Figure 6: *Effect of BSP on the biliary concentration of benzophenones after administration of diazepam (300 mg/kg, i.p.). The diazepam was given 1 hour before the BSP. Each point is the mean ± SE of 4 rats. The diazepam was given at 0 hour.*

TABLE I

EFFECT OF BSP ON THE BILIARY EXCRETION OF DIAZEPAM METABOLITES IN RATS*

Dose of BSP (mg/kg)	Total Benzophenones Measured (mg/kg) in 4 hours 50 mg/kg	100 mg/kg	150 mg/kg	300 mg/kg
0	25	31	48	72
60	19	29	34	48
120	16	21	35	28

* Each value is the mean of 4 rats

Table I illustrates the effect of BSP on the total amount of benzophenones measured within the 4-hour collection period. In control rats, the total amounts of benzophenones measured represent 50, 31, 32 and 24% of the respective doses of diazepam administered. It can be seen that both the 60 mg/kg and the 120 mg/kg of BSP reduced somewhat the total amount of benzophenones when 50, 100 and 150 mg/kg of diazepam were administered. However, the most marked effect was observed when 300 mg/kg of diazepam was given; in this case, there was a 60% reduction in the amount of benzophenones measured.

Effect of Diazepam on the Biliary Excretion of BSP

These studies were carried out using two doses of BSP (60 or 120 mg/kg). Four different doses of diazepam (50, 100, 150 or 300 mg/kg) were utilized. The BSP excreted into the bile was analyzed for five consecutive 15-minute periods. In all of these experiments, the diazepam was administered 1 hour before the administration of the BSP.

Figure 7 depicts the effect of diazepam on the excretion of BSP, 60 mg/kg. The amount of BSP excreted into the bile has been expressed on a mg/kg body weight basis. Since each collection period was analyzed separately, the values given in the figure represent the amount of BSP excreted per 15 minutes. With this dose of BSP, the biliary excretion rate reached its maximum by 30 minutes (about 20 mg/kg/15 min) in control rats. The rate was much lower at 45 minutes and, by 75 minutes, biliary excretion was near termination.

When diazepam was administered, the maximal BSP excretion rate attained was lower (7 to 15 mg/kg/15 min versus 20 mg/kg/15 min); a dose-dependent effect seemed to exist. Even a dose of 50 mg/kg of diazepam was capable of depressing BSP excretion rate. As one would expect, this decrease in maximal excretion rate resulted in a more prolonged excretion of BSP. At 75 minutes, the rats given 100-300 mg/kg of diazepam were still excreting BSP at rates of 5-7 mg/kg/15 min.

This effect of diazepam on the biliary excretion of BSP was even more evident when the BSP dose was increased to 120 mg/kg. These data are shown in Figure 8. With this dose of BSP, the peak rate of excretion occurred at 45 minutes, yielding a value of about 27 mg/kg, in control rats. With doses of diazepam ranging from 50 to

300 mg/kg, the amount excreted at 45 minutes was about 33% of that measured with BSP in control animals. The maximal BSP excretion rate attained with diazepam treatment never exceeded 12 mg/kg/15 min. Again, a dose-related response was observed, with 300 mg/kg of diazepam exhibiting the most marked effect. With this dose of BSP, it is not possible to determine the effect of diazepam on the duration of excretion since the experiment was terminated before excretion had terminated in the control animals.

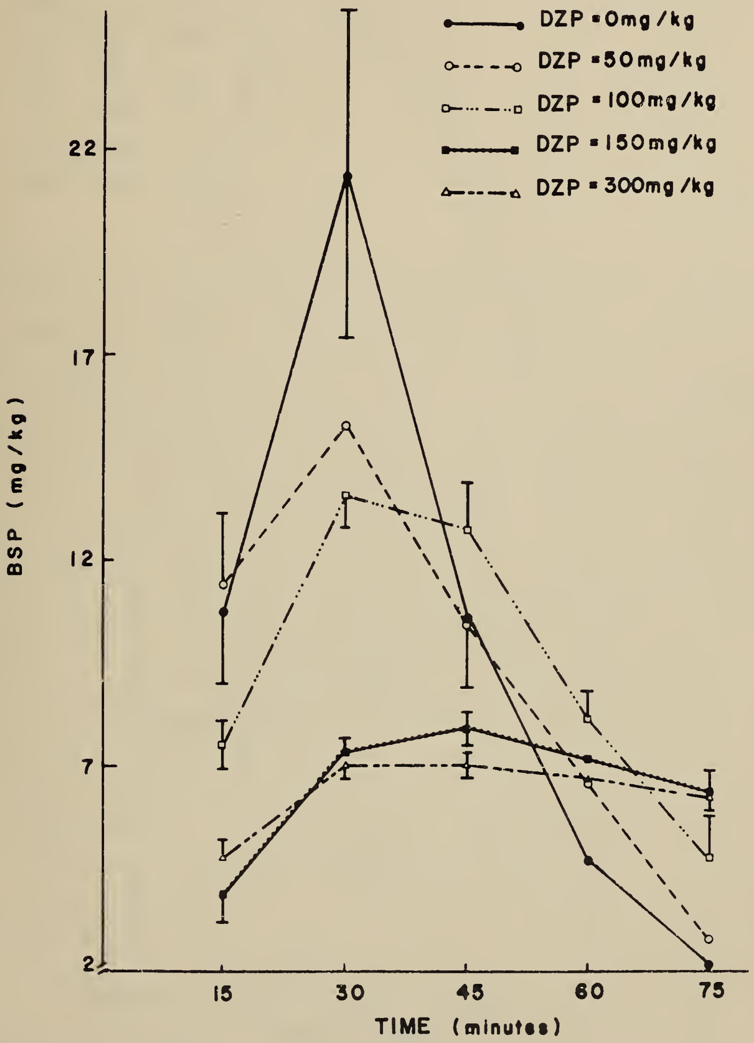

Figure 7: *Effect of diazepam on the biliary excretion of BSP (60 mg/kg, i.v.). The diazepam was given 1 hour before the BSP. Each point is the mean ± SE of 4 rats. The BSP was given at 0 minute.*

If one measures BSP concentration in the bile, with 60 mg/kg of BSP, a peak value of about 16 mg/ml is reached at 30 minutes in control rats (Figure 9). All four doses of diazepam were capable of diminishing the peak concentration of BSP by about 50%. Again, at 75 minutes, higher concentrations of BSP were detected in the diazepam-treated animals than those seen in control rats. The diazepam-induced lowering of BSP concentrations was also evident when the BSP dose was increased to 120 mg/kg (Figure 10). After diazepam, the peak concentrations never rose above 10 mg/ml, at 45 minutes, although peak values of about 16 mg/ml were attained in control rats.

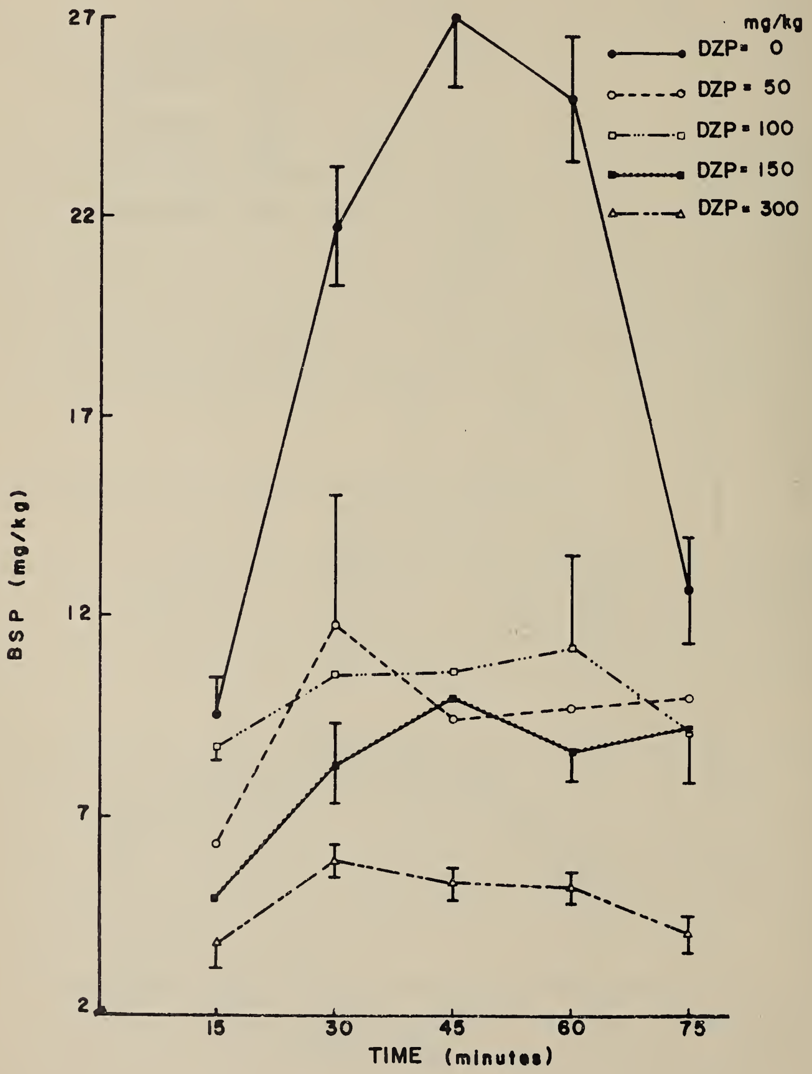

Figure 8: *Effect of diazepam on the biliary excretion of BSP (120 mg/kg, i.v.). The diazepam was given 1 hour before the BSP. Each point is the mean ± SE of 4 rats. The BSP was given at 0 minute.*

TABLE II

EFFECT OF DIAZEPAM ON THE BILIARY EXCRETION OF BSP IN RATS*

Dose of Diazepam (mg/kg)	Total BSP excreted (mg/kg) in 1.25 hours		Bile flow (ml/kg) in 1.25 hours	
	60 mg/kg	120 mg/kg	60 mg/kg	120 mg/kg
0	49	96	5.5	6.7
50	46	47	6.7	4.5
100	47	50	6.9	4.1
150	32	41	4.2	4.6
300	31	24	4.8	3.2

*Each value is the mean of 4 rats

In Table II, the total biliary excretion of BSP during the 1.25 hours is summarized. It can be seen that with control animals receiving 60 or 120 mg/kg of BSP, 49 and 96 mg/kg (82 and 80% of the dose) were excreted within 1.25 hours. When diazepam was given, there was little effect on the total amount of BSP excreted during the total time of collection until the dose of diazepam was raised to 150 or 300 mg/kg, when 60 mg/kg of BSP was the test dose administered. However, when 120 mg/kg of BSP was given, even 50 mg/kg of diazepam reduced the total amount of BSP excreted by about 50%. With 300 mg/kg of diazepam, the amount of BSP excreted was less than 25% of normal. The last two columns in Table II illustrate the effects of diazepam on bile flow; it is known that diminished bile flow can decrease BSP biliary excretion. It is evident that when 60 mg/kg of BSP was utilized, there was little effect on bile flow (total bile volume was 87% of control). However, when the 120 mg/kg dose of BSP was used, there was a marked reduction in bile flow (total bile volume was 47% of control). These reductions in bile flow alone cannot account for the reduction in total BSP excreted seen when diazepam was given. The BSP concentration was diminished by diazepam treatment. This indicates that the diminution in BSP excretion, particularly when 120 mg/kg of BSP was used, was due to an effect on the excretion of BSP itself and not only an effect on bile flow.

The data in Figures 9 and 10 suggested that the BSP excretory capacity of the liver had been diminished by diazepam treatment. Whether the dose of BSP administered was 60 mg/kg or 120 mg/kg, the peak concentration of BSP after diazepam was always about 10 mg/ml. The peak concentration of BSP under conditions of biliary transport maximum is over 15 mg/ml.[14, 15] Consequently, an experiment was performed in which BSP Tm was measured by the method of Klaassen and Plaa.[16] The Tm value for control rats was 1.40 ± 0.07 mg/kg/min, whereas the value for rats treated with diazepam (300 mg/kg) was significantly ($p<0.05$) reduced to 0.80 ± 0.11 mg/kg/min. A similar experiment was carried out using BSP-glutathione instead of BSP. This substance is the major conjugate of BSP appearing in rat bile and accounts for more than 70% of the BSP excreted into the bile.[1] In control rats, the Tm for BSP-glutathione was 1.43 ± 0.11 mg/kg/min. In diazepam-treated rats, the value was 1.69 ± 0.03 mg/kg/min, which was not significantly different from the control value. Therefore, diazepam reduces the Tm of BSP but not that of its conjugated metabolite.

The acute effects of diazepam, given parenterally, on BSP excretion would suggest that the acute pretreatment with this agent should lead to BSP retention in the plasma. An experiment was performed to test this hypothesis. Rats were given varying doses of diazepam intraperitoneally. One hour later, the animals were given BSP, 60 mg/kg, intravenously and blood samples were obtained 30 minutes later.

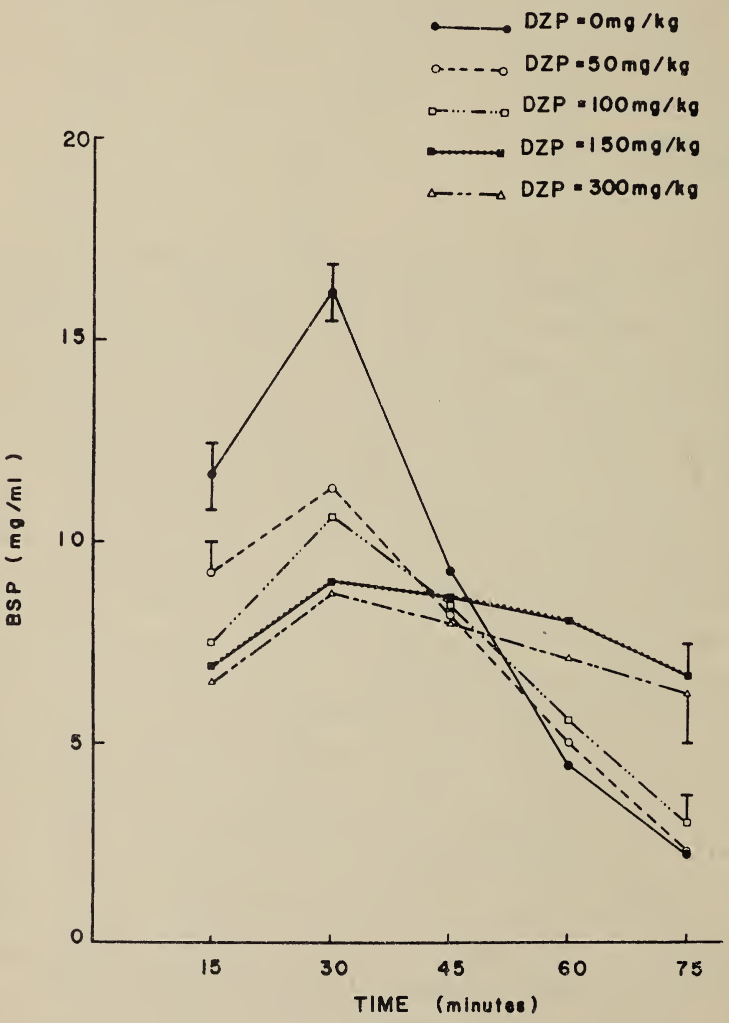

Figure 9: *Effect of diazepam on the biliary concentration of BSP (60 mg/kg, i.v.). The diazepam was given 1 hour before the BSP. Each point is the mean ± SE of 4 rats. The BSP was given at 0 minute.*

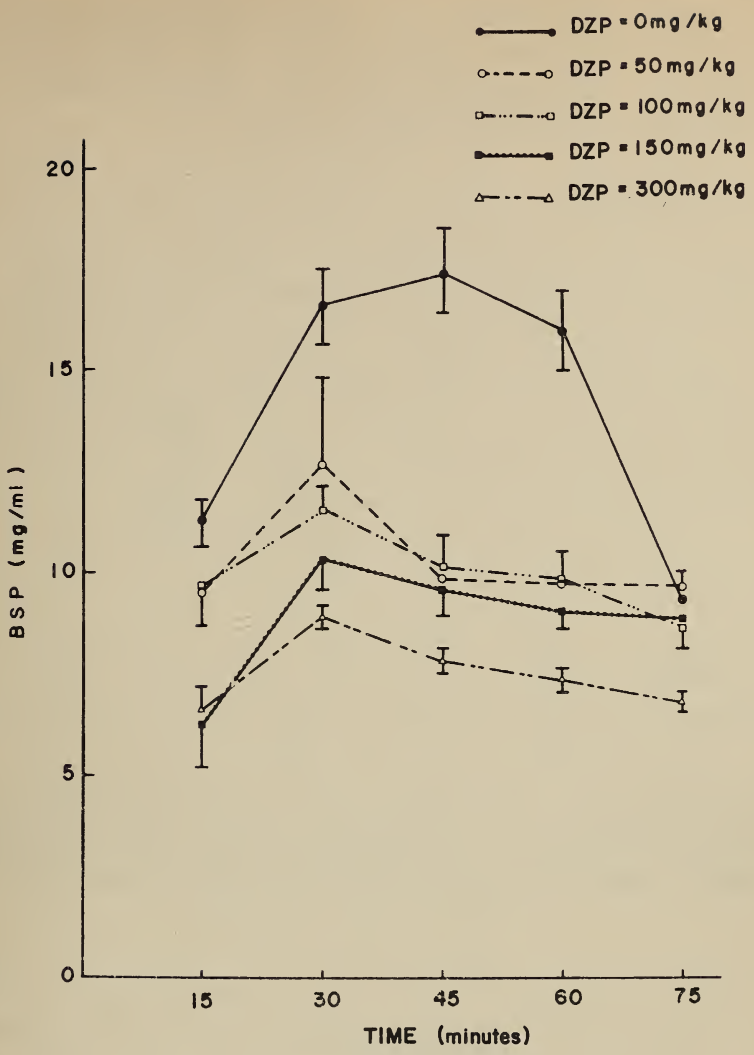

Figure 10: *Effect of diazepam on the biliary concentration of BSP (120 mg/kg, i.v.). The diazepam was given 1 hour before the BSP. Each point is the mean ± SE of 4 rats. The BSP was given at 0 minute.*

The plasma was analyzed for BSP content after alkalinization with 0.01 N NaOH. The results are shown in Figure 11. Doses of diazepam commencing with 50 mg/kg resulted in plasma BSP retention (control, 0.51 ± 0.04 mg/100 ml versus diazepam, 0.76 mg/100 ml), maximal retention was observed when 300 mg/kg of diazepam was given (4.78 ± 0.69 mg/100 ml). However, diazepam (100 mg/kg) did not cause abnormal BSP retention when BSP-glutathione (76.1 mg/kg) was given instead of BSP. In control rats, the 30-minute plasma concentration of BSP-glutathione was 1.2 ± 0.2 mg/100 ml; in diazepam-treated animals, the value was 1.0 mg ± 0.2 mg/100 ml.

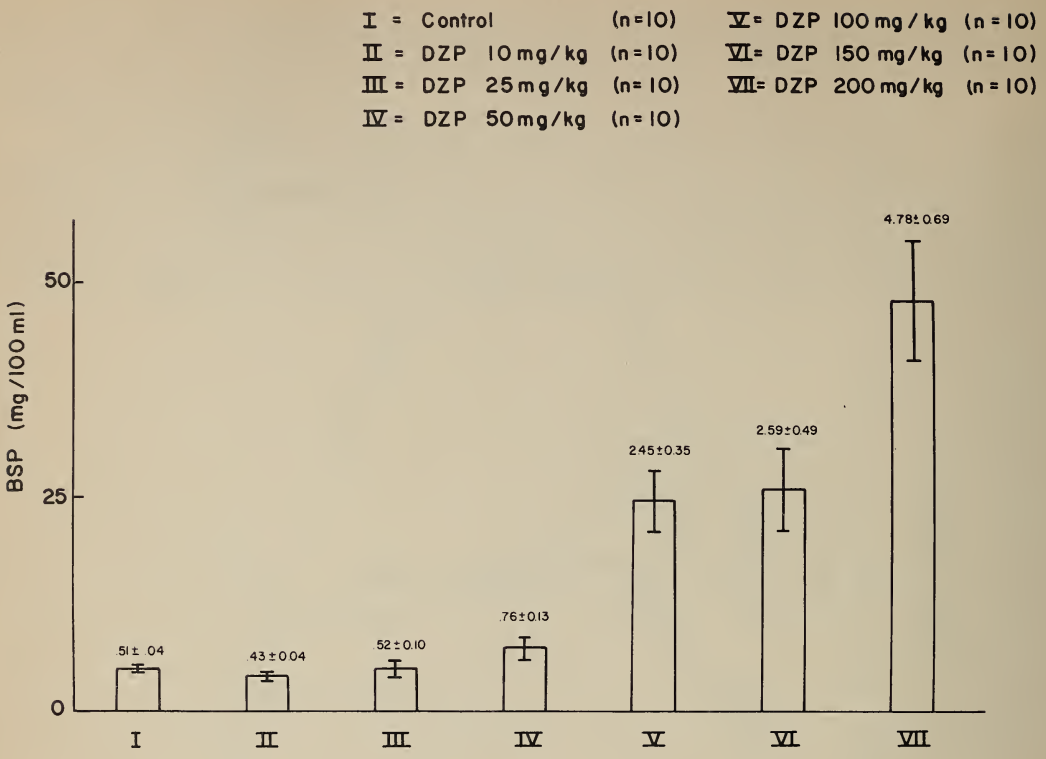

Figure 11: *Effect of diazepam on the plasma retention of BSP (60 mg/kg, i.v.). The diazepam was given 1 hour before the BSP. The plasma BSP concentration was determined 30 minutes after the administration of BSP. Each value is the mean ± SE of 10 rats.*

DISCUSSION

Besner *et al.*[11] have recently demonstrated that diazepam itself is not excreted into the bile of rats, but that the glucuronides of its metabolites, primarily 4'-hydroxydesmethyldiazepam and 3-hydroxydiazepam, do appear in bile. Since the molecular weights of these conjugates would be around 460, the bile could represent an important route of excretion for these substances in the rat.

The data in Table I indicate that such is the case. The amounts of benzophenones measured in control rats ranged from 25 to 50% of the dose of diazepam administered. The highest percentage excreted occurred with the 50 mg/kg dose of diazepam. It should be noted that these percentages most likely represent the lowest estimates of biliary excretion, because (1) bile was collected for only 4 hours and the excretion of diazepam metabolites was still going on, and (2) absorption of diazepam may not have been complete since the diazepam was administered intraperitoneally in suspension.

The data in Figures 5 and 6 show that, as the dose of diazepam was increased from 50 to 300 mg/kg, the peak concentration of benzophenones rose from a peak of 2 mg/ml to one of 3.6. This suggests that even at 300 mg/kg, saturation of the biliary excretory system may not have occurred.

However, since with 300 mg/kg, the benzophenone biliary concentration remained above 3 mg/ml for the entire 4 hours, it would appear that this concentration may be approaching the maximum attainable. If this is so, one could estimate that the maximal rate for biliary excretion of diazepam metabolites would be larger than 20 mg/kg/hour (0.33 mg/kg/min) in the rat.

It was not possible to quantify the amounts of diazepam or its metabolites in the blood of these rats during these experiments. In other experiments using 150 mg/kg of diazepam, the plasma concentrations were found to range from 0.03 to 0.05 mg/ml. Biliary peak concentrations of benzophenones observed are equivalent to about 1 to 4 mg/ml of diazepam. Therefore, it appears that the bile to plasma ratios of the diazepam metabolites are most likely greater than 100.

Finally, it was demonstrated that another organic anion, BSP, could interfere markedly with the biliary excretion of diazepam metabolites. The decrease in biliary excretion was more evident when the dose of BSP was increased. For all of the above reasons, it appears that the conjugated metabolites of diazepam meet the criteria established for the biliary excretion of organic anions.[1, 2]

A second objective of the study was to see whether diazepam could interfere with the biliary excretion of BSP. The data in Figures 7 and 8 show that indeed diazepam administration does decrease BSP excretion in a dose-related manner. However, certain characteristics of the data suggest that the interference does not seem to be merely a competition for a site for transport. If one assumes that the relative affinities of the substances concerned for the transport site are independent of their respective concentrations, one would expect that increasing doses of diazepam would have a more marked effect on the lower dose of BSP than on the higher dose. This was not the case. The smallest dose of diazepam seemed to have a more marked effect on the higher dose of BSP than it did on the lower dose of BSP when excretion rates were measured (Figures 7 and 8).

These data suggested that the biliary excretory capacity for the excretion of BSP was decreased by diazepam treatment. The experiment designed to verify this conclusion demonstrated that diazepam indeed did lower the apparent Tm for BSP excretion, but not the apparent Tm for BSP-glutathione excretion. These observations suggest that diazepam interferes with the hepatic uptake, the conjugation step with glutathione, or the hepatic storage of BSP. These parameters remain to be investigated. Therefore, while it is possible that metabolites of diazepam can interfere with the biliary excretion of BSP, it is not clear whether diazepam or its metabolites might not interfere with the other steps involved in BSP elimination.

Finally, it has been shown that, regardless of the mechanism, the acute administration of diazepam in high doses given parenterally can lead to BSP retention in the plasma of rats. It would be of interest to see whether a similar occurrence exists after chronic oral treatment with diazepam. If so, one could envision the possibility of this leading to false positive liver function tests in subjects treated with diazepam.

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Research on the Non-Medical Use of Drugs: The Challenge to Pharmacologists and Toxicologists

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ABSTRACT An assessment of available Canadian statistics on the non-medical use of drugs indicates that alcohol consumption and tobacco use still involve serious personal and social consequences. The misuse of physician-prescribed mood-modifying drugs is not only hazardous for the particular people involved, but also shows a positive correlation to the use of psychotropic agents by their children. In addition to the toxic effects of mood-altering drugs such as narcotics, amphetamines, barbiturates, one must now consider the increasing multiple-drug use and its consequences.

Pharmacologists and toxicologists have carried out extensive research on the above types of drugs in their respective areas of expertise. Behavioural pharmacologists and toxicologists have been able to make significant advances in developing methodology and instrumentation for investigating mood-altering drugs. The challenges posed to pharmacologists and toxicologists are to address their research pursuits to some of the pressing issues associated with non-medical drug use and to seek the involvement of behavioural researchers in their work in carrying out investigations with broader and perhaps more meaningful approaches.

Three topics will be covered in this presentation: an overview of recent work to appraise the present problems in Canada associated with drugs of abuse; a discussion of some of the studies presently in progress on the behavioural aspects of non-medical drug use and comments on the increased involvement of pharmacologists and toxicologists in the broader issues of drug abuse research.

EPIDEMIOLOGY

The extent of non-medical drug use in a society and the consequences resulting from such use can never be perfectly known. Even if they could, it would quickly become out-dated information, as experience has shown drug use patterns to be episodic and volatile. Thus, in order to obtain a reasonably accurate picture of the drug scene at

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any particular time, the optimum strategy is to take many different types of statistics and search for those which seem to be consistent. This is the strategy which we intend to pursue to acquire up-to-date information on the extent, nature and consequences of drug abuse and to relate our programs and priorities to the real problems arising from the non-medical use of drugs in our Canadian society.

At this particular point in time, however, our indicators of the extent and consequences of non-medical drug use in Canada are not able to give us as complete and accurate a picture of the current drug scene as we would like. Not only are there some serious gaps in our information, such as an absence of systematic analysis of the content of "street drugs" not obtained through police channels, but there is also a serious time lag before some potentially useful information becomes available to us (e.g., Poison Control statistics[1]). We are presently working to correct some of these deficiencies in order to be able to provide the most accurate, up-to-date picture possible of the current drug scene in Canada. Given these limitations, and others not mentioned, what then is the current status of the Canadian drug scene?

There can be little doubt that in terms of numbers involved and personal and social consequences, alcohol and tobacco are still, and are likely to continue to be for some time to come, the most costly non-medically used drugs in Canada. In 1970 it was estimated that Canada's drinking population numbered 12 million, 720,000 of whom were consuming amounts hazardous to their health.[2] Recently it was estimated that alcohol abuse cost Ontario taxpayers $134 million in just one year for illness and disruption attributed directly to alcoholism.[3] There is evidence that over 50 per cent of fatally injured drivers and pedestrians have demonstrable blood alcohol levels at the time of the accident.[4] Alcohol has also been implicated in many types of mortalities: 43 per cent of accidental fire deaths, 25 per cent of deaths due to falls, 38 per cent of cirrhosis deaths, 22 per cent of peptic ulcer deaths, 18 per cent of suicides, 15 per cent of pneumonia deaths, and 16 per cent of deaths due to cancer of the upper digestive and respiratory tracts as well as 5 per cent of deaths due to heart or artery disease.[3] Moreover, a series of recent studies of drug use among metropolitan Toronto high school students leads to the conclusion that "alcohol now appears to be the drug of increasing popularity", which probably means that Canada cannot expect any immediate relief from the consequences of alcohol abuse.[5]

Although recent studies indicate that the proportion of smokers in Canada is decreasing, almost half of the Canadian population over 14 years of age smoke tobacco, and the majority of these do so regularly (47% of the males and 32.4% of the females).[6] The social and personal costs of such a behaviour pattern is extremely difficult to estimate, but one fairly recent source suggested that in terms of certain identifiable consequences such as lung cancer, coronary disease, chronic bronchitis, emphysema, and fires caused by smoking, it amounts to almost 400 million dollars.[7] It is probable that an estimate of more than $500 million would not be unreasonable today. This estimate does not, of course, cover the misery and agony of the suffering that tobacco-linked diseases inevitably entail.

Another type of non-medical drug use which should be of current concern to us in terms of numbers involved and possible costs is the misuse of physician-prescribed mood-modifying drugs by adults. A recent Toronto study by Cooperstock and Sims, for instance, suggests that prescriptions for mood-modifying drugs constitute one-quarter of all prescriptions dispensed to a non-hospitalized urban population.[8] Evidence from two recent American surveys of national samples outlines a similar picture. These studies found that about one-quarter of U.S. adults use one or more kinds of psychotropic drugs. Nearly half of the United States adult population report use of a psychotropic drug at some time. Cumulative use of tranquillizers over the last decade has shown a steady increase from about 7 per cent of the population in 1957 to about 27 per cent in 1967.[9] There is no particular reason to believe that the Canadian situation is much different. This type of drug use, in addition to being hazardous for the particular individuals involved, has other indirect consequences as suggested by the finding of a strong, positive relationship between use of psychoactive drugs by parents and use of psychoactive drugs by their children.[10]

This discussion of the extent of and possible hazards associated with, the use of alcohol, tobacco, and other mood-modifying drugs by adults should not be allowed to obscure our concern with other drugs of abuse. In particular, we should not lose sight of the personal and social costs related to the injection of opiate narcotics, amphetamines and other substances by young people in Canada. It has been suggested, for instance, that the increased incidence of viral hepatitis in recent years is largely due to its spread among youths in the drug culture and that in fact, drug abuse has become the major determinant of the pattern of viral hepatitis in North America.[11] It has also been suggested that various other disorders such as tetanus, cardiovascular-respiratory failure, pulmonary fibrosis, edema and endocarditis are also associated with heroin addiction.[12] In addition, although there are no accurate statistics available for Canada, it is probable that heroin abuse is costly in terms of increased crime, criminal justice expenditures, and loss of manpower and productive capacity.[13] Thus, the recent upward trend in the amount of heroin abuse in Canada indicated in Federal statistics [1, 14, 15] is particularly worrisome.

These statistics for 1971 and 1972 also suggest a rise in the amount of amphetamine and cocaine abuse, although other sources[5, 16] report a reversal of this trend with respect to amphetamines. Whatever the case, the appearance of new potentially deadly substances on the illicit market, such as PMA (4-methoxyamphetamine) early this year, should warn us against decreasing our vigilance.

Federal statistics[1, 14, 15] and other sources[5] suggest a leveling off in the use of cannabis and other psychedelic drugs in Canada. Federal Poison Control statistics[1] and the recent survey of student drug use in Toronto[5] also indicate a leveling off in the amount of solvent abuse. The picture with respect to barbiturate use is somewhat conflicting with some sources[1, 5] suggesting an increase, and others[14, 15] a decline. However, it has been suggested that the current epidemic of barbiturate abuse in the United States may not augur well for Canada[17].

Finally, one type of non-medical drug use about which we have little, if any, accurate Canadian data, but about which there is a growing suspicion of a rapid increase in incidence, is multiple, or poly-drug use. This should be of particular concern to us because of the largely unknown, but potentially serious consequences of the interaction of various types of substances. From a research point of view, it means that we must increasingly focus on the study of the fundamental causes of drug use rather than on the causes of using any one drug. Doing so, requires a heightening of the level of research sophistication, as well as an increasing emphasis on inter-disciplinary work.

BEHAVIOURAL PHARMACOLOGY AND TOXICOLOGY

Behavioural pharmacology is a diverse, but rapidly developing area of biological and medical science concerned with the analysis of those mechanisms whereby drugs influence behavioural processes and brain function. The precision and sensitivity of new techniques developed recently for the experimental analysis of behavioural processes has made the behavioural actions of drugs amenable to scientific investigation. Despite an extensive literature, however, it is still possible to draw but few general conclusions concerning the behavioural effects of drugs. It is equally disturbing to realize that, at a physiological-biochemical level, the mechanism of action of any drug on the central nervous system cannot completely be described.

The clinical and experimental aspects of behavioural pharmacology are intimately related. Experimental data pertaining to drug-induced changes in behaviour and brain function have assisted the clinician in his appreciation of the manner in which various drugs may affect an aberrant condition or provide information related to selective toxicity or other hazardous effects associated with particular drugs. Conversely, clinical observations derived from situations in which a behaviourally-active drug has been found to alter a patient's abnormal or psychiatric condition, have frequently stimulated basic research employing techniques for controlled experimentation in neurochemistry, neurophysiology and behaviour.

However, as Weiss and Laties[18] have pointed out, it should be noted that extrapolating findings from one species to another is not without difficulty. There are species differences, not only in absorption, distribution and metabolism of drugs, but also in drug-induced behavioural processes attributable to learning and prior experience.

Behavioural Pharmacology: Objectives

Four general objectives are characteristic of contemporary research dealing with the manner in which drugs influence behavioural processes:

1. The use of reliable and well-controlled behavioural techniques to analyse and describe the selective effects of drug action.
2. The use of drugs to afford a method of analysing complex behavioural processes.
3. The use of suitable behavioural procedures for screening newly synthesized drugs in order to determine their potential usefulness for human clinical application.
4. The use of behavioural techniques to determine possible hazards associated with particular drugs including the description of toxic side effects and probability of compulsive abuse or dependence resulting from chronic or repeated drug administration.

Behavioural Toxicology

Weiss and Laties note, "Many studies of the behavioural effects of drugs can be conceived of as attempts to determine selective toxicity in the context of a therapeutic aim".[19]

Current awareness of various forms of environmental pollution on the one hand, and abnormal behaviour or confusional states associated with non-medical drug usage on the other, are undoubtedly widening the field of toxicology and, within it, the role of behavioural toxicology. Most toxicological studies have dealt with the classic pharmacology/toxicology approaches. Few experiments are available, however, to assess the substantial behavioural effects of various industrial poisons, air contaminants and drugs at concentrations lower than those needed to produce overt pathology. One or two examples mentioned by Weiss and Laties[18] can be cited which reveal the seriousness of the problem and a relative lack of information along these lines.

Armstrong *et al*[20] investigated the effects of mercury vapour on the behaviour of pigeons working under a multiple schedule of food reinforcement. Reversible changes in the behavioural baselines were observed before the investigators could detect gross changes in behaviour or overt pathology. Hanson, Witoslawski and Campbell[21] examined the effects of pheniprazine, a MAO inhibitor, on red-green color blindness in pigeons. The drug had previously been shown to cause this type of visual defect in humans. Hanson *et al* trained pigeons to peck a key when it was transilluminated with light of either 570 or 610, but not 550, 590 or 630 millimicrons. Chronic dosing disrupted the discrimination at dosages which did not interfere with overall rates of responding. Withdrawal of the drug led to a recovery of discrimination performance.

Perhaps these two examples are sufficient to illustrate the relatively subtle effects of toxic agents, even though from a psychological standpoint, these effects constitute a major impairment of behavioural function. An advanced technology is needed urgently for the behavioural evaluation of toxic agents.

EXPERIMENTAL APPROACHES TO THE PROBLEMS OF DRUG DEPENDENCE

Drug dependence must be viewed as a complex phenomenon in both the bio-medical and socio-behavioural context. Animal investigation of drug dependence as an approach to the understanding of human drug addiction runs a serious risk of over-simplication.[22] Nevertheless, there are pharmacological and behavioural aspects of addiction that can be reproduced in experimental animals and call for detailed study.

A drug which serves as a controlling consequence for behaviour leading to its administration may be conceptualized as a reinforcer. Behavioural dependence is then said to occur if a previously untrained animal adopts and repeatedly emits a pattern of behaviour which is instrumental in producing prompt administration of the drug.[23, 24] Recent experiments, employing chronic catheterization techniques for intravenous drug administration,[25, 26, 27] have shown that discrete response contingent infusions of a number of different drugs serve to establish and maintain sequences of behaviour which are effective in producing successive infusions of the drug in question.

The largest body of evidence on drug self-administration which falls within the operant conditioning paradigm relates to opiate self-administration by rodents and monkeys. Experiments have dealt, for example, with nalorphine-induced changes in morphine self-administration in rhesus monkeys.[28]

It should also be noted that other drugs including the barbiturates,[29, 30] the amphetamines and their chemically-related derivatives,[23, 31, 32] cocaine,[33] as well as ethanol and nicotine have been shown to serve as reinforcers.

Data are now available therefore to justify a systematic examination of those factors governing the ability of drugs to serve as reinforcing consequences for self-administration behaviour. It is essential, however, that these behavioural investigations of drug self-administration behaviour should be accompanied by concurrent neurochemical and neurophysiological investigations of states of dependence and withdrawal if a comprehensive account of the processes of addiction is to emerge.

NEUROCHEMICAL AND PHYSIOLOGICAL CORRELATES OF DRUG ACTION

Intensive research is being undertaken to provide a coherent account of the biochemical and physiological changes in the nervous system which are known to accompany drug-induced changes in behaviour. Russell[34] has provided a number of useful comments concerning research strategy with respect to methodological levels of analysis which deserve careful consideration. He identified two types of hypothesis which are likely to be formulated.

First, a drug serves as an independent variable and a particular process serves as the dependent variable. For example, a given drug may affect a measureable neurochemical event, it may affect a recorded neurophysiological response, or it may alter the occurrence of an identifiable sequence of behaviour. In each case, recourse to an alternative level of analysis is unnecessary to characterize the action of the drug upon the chosen dependent measure. Beyond a descriptive level, an eventual integration of knowledge concerning a drug's biochemical, physiological and behavioural effects should be possible.

Second, it is apparent that a drug may serve as an independent variable in the analysis of interactions between neurochemical events and behaviour or between neurophysiological processes and behaviour. For example, a particular drug may affect a known neurochemical process, changes in which are reflected in a specific behavioural activity. This kind of hypothesis directed towards an understanding of the neurochemical and neurophysiological correlates of drug-induced changes in behaviour, emphasizes a reciprocal relationship between neurochemical and behavioural events. Russell proposes a simple model of the type:

$$\begin{tikzcd} \text{DRUGS} \arrow[r] & \text{NEUROCHEMICAL EVENTS} \arrow[r, shift left, "1"] & \text{BEHAVIOUR} \arrow[l, shift left, "2"] \end{tikzcd}$$

The neurochemical-behavioural interaction is a reciprocal one in the sense that (1) drugs as chemical stimuli produce changes in neurochemical processes which may in turn alter behaviour and (2) behaviour may produce changes in neurochemical processes thereby modifying the manner in which these processes are affected by a drug treatment.

DISCUSSION

In this paper we have described an overview of the nature and extent of the non-medical use of drugs in Canada and noted some of the problems associated with the abuse of mood-altering drugs. This material was presented to give a perspective of the areas of research to which we must address our attention and interest; as responsible scientists we must pool our resources to help resolve some of the pressing issues associated with the abuse of drugs.

Secondly, a description of a related discipline(s) namely, behavioural pharmacology and toxicology, was given to suggest the need for a broadening of the scope of researchers involved in pharmacology and toxicology studies.

We would like to suggest that two challenges to pharmacologists and toxicologists interested in studying the problems associated with the non-medical use of drugs are:

1. To address their research pursuits to some of the pressing issues associated with drug abuse.

2. To broaden the scope of their research investigations by incorporating a multi-disciplinary approach. Scientists with training in pharmacology and toxicology must encourage greater collaboration with those studying similar problems within the behavioural sciences.

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